A STUDY OF SOME FACTORS AFFECTING THE DISTRIBUTION OF DRUGS ACROSS THE EX-VIVO PERFUSED HUMAN PLACENTA

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A STUDY OF SOME FACTORS AFFECTING THE DISTRIBUTION OF DRUGS ACROSS THE *EX VIVO* PERFUSED HUMAN PLACENTA

Clayborne M. Monk

ABSTRACT

A Modification of the *ex vivo* placental perfusion system designed by K. E. Krantz, *et al*. (1), in which both maternal and fetal circuits of the placenta are perfused, was employed in order to study the rate and extent of transfer of antipyrine (ANT), sulfanilamide (SNL), sulfadimethoxine (SDM), N-4-acetyl-sulfanilamide (ASL), and sulfobromophthalein (BSP) across the human placenta. The dispositions of dextrose (GLU), which was added to the perfusate as a nutrient, blue dextran (BD), which was used as a volume marker for the fetal circuit, and low molecular weight dextran (DEX), which was added to the fetal circuit as an osmotic colloid, were also studied in this preparation.

Poor availability of placentas suitable for perfusion necessitated the design of experiments in which the disposition of several of the test substances were studied in a single perfusion experiment, thus requiring the development of methods for simultaneous spectrophotometric analysis of the test substances.

The perfused placentas were metabolically active, as evidenced by disappearance of GLU from the perfusates, and by the appearance of lactate and pyruvate in the perfusates at rates which are comparable to rates reported by other investigators.

Fetal to maternal bulk flow of fluid occurred during

perfusion of each placenta which, to some extent, is to be expected due to a normal fetal to maternal hydrostatic pressure gradient. Bulk flow rates greater than 2 ml/min were accompanied by increased perfusion pressures and increased fetal capillary permeability to BD or DEX. Such circumstances may be the result of hypoxia and/or lack of globulins in the perfusate.

Clearances of ANT, SNL, SDM, ASL, BSP, and GLU, between the placenta and the maternal or fetal circuit, were flow rate limited as evidenced by the observation that no differences existed in the initial rates or disappearance of the various test substances from the circuit to which they were initially added.

On the other hand, clearances of the test substances across the placenta were dependent on the extent of placental uptake by, and/or the diffusional resistance of each substance through the placenta, as evidenced by significant differences in the rates of appearance of the test substances in the circuit opposite to which they were initially added.

There were no qualitative differences in the relative disposition rate characteristics of the test substances, in spite of variation in fetal to maternal bulk flow rates of fluid during the perfusion experiments.

Placental uptake of BSP and ASL was much higher than expected solely on the basis of lipid solubility and degree of ionization of the compounds. This greater uptake for BSP and ASL is probably due to tissue binding.

The data from these experiments show that the kinetics of human placental transfer is best described by models which

represent the placenta as two or more compartments. Due to the problem of variable bulk flow, the data were inadequate for the precise definition of a model.

The tendency for attainment of a maternal to fetal gradient for all test substances was observed in each experiment. Such gradients are often seen in vivo with substances which are not actively transported and may be due, in part, to fetal to maternal bulk flow in addition to bidirectional diffusion of the drug.

Further work is needed to improve the viability of the ex vivo perfused human placenta. Once improvement is accomplished, the use of this preparation will enable a better definition of the effect of the placenta on the rate and extent of drug transfer to the fetus.

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(1) K. E. Krantz, T. C. Panos, and J. Evans, Amer. J. Obstet. Gyn., 83, 1214, (1962).

To

My Parents

Thomas N. and Lillian G. Monk

and

My Grandmother

Rosita R. Monk

who have always been sources of

encouragement and inspiration to me

To

My Wife

Anita E. Monk

Who, in addition to the help she gave in proof-

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To

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Susan E. and Michael T. Monk

who provided the motivation for this study

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LIST OF ABBREVIATIONS

ANT	Antipyrine
ASL	N-4-Acetylsulfanilamide
BD	Blue Dextran-2000
BSP	Sulfobromophthalein
DEX	Low molecular weight dextran
GLU	Dextrose
LDH	Lactate dehydrogenase
NAD	Nicotinamide adenine diphosphonucleotide
NADH	Nicotinamide adenine diphosphonucleotide, reduced
SDM	Sulfadimethoxine
SNL	Sulfanilamide

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INTRODUCTION

Nature of the Problem

Ours is a drug-oriented society from which pregnant women are not exempt. Statistics gathered in surveys of drug use by pregnant women, during their first trimester and during the whole pregnancy, are presented in table I. The survey in Scotland and presumably the survey in Texas and Wisconsin were made subsequent to the late 1961 reports of thalidomide teratogenicity, however, little or no apparent difference in the numbers of drugs used by women surveyed before and after the thalidomide reports is seen by examination of the data presented in table I.

The need for drug therapy during pregnancy may or may not be related to the state of pregnancy. Such complications of pregnancy as nausea, vomiting, anemia, threatened abortion, hypertension, toxemia of pregnancy, and excessive weight gain, are commonly treated with drugs. Although the drugs used to treat these complications represent a significant fraction of those drugs received by pregnant women, the majority of drugs received are intended to treat various acute and chronic ailments which are unrelated to the state of pregnancy (3).

In addition to those drugs received by pregnant women before labor, various gaseous and local anesthetics, hypnotics and sedatives, analgesics, tranquilizers, and skeletal muscle relaxants are commonly administered during labor and delivery to ease pain and discomfort (5,6).

Maternally administered drugs may produce harmful effects on the conceptus at any time between fertilization and birth (7).

TABLE I

Drug Usage During Pregnancy

Location of Survey (Reference)	PERIOD OF PREGNANCY				
	FIRST TRIMESTER			ENTIRE PREGNANCY	
	New York(1)	Scotland(2)	Oakland(3)	Scotland(2)	Texas and Wisconsin(4)
Period of Survey	1953-1957	1962-1964	1960-1961	1962-1964	-----[a]
Number of Women Surveyed	798	1369	3072	1369	240
Types of Drugs Reported[b]	Rx, OTC[c]	Rx[d]	Rx	Rx[d]	Rx, OTC
Average Number of Drugs Taken per Woman	0.97	1.03	3.6[e]	3.5	5.4
Women Who Took No Drugs (%)	48	44	7.9	2.6	-----[f]
Women Who Took One Drug (%)	28.2	29.7	18.3	18.8	-----
Women Who Took More Than Five Drugs (%)	1.7	2.8	20.8	16.0[g]	-----

[a] Not stated, survey published 1967.
[b] Rx = prescribed drugs; OTC = self medicated drugs.
[c] Vitamins, antacids, salicylates, caffeine, and laxatives excluded.
[d] 65% of women surveyed also took OTC medication.
[e] Includes drugs taken within the three months prior to the last menstrual period.
[f] ----- = not reported.
[g] More than ten drugs were taken by 3.9% women surveyed.

The specific adverse effect produced by a particular drug depends on the gestational age of the conceptus when the drug is taken, the dose of the drug, and the duration of therapy. The effect produced may be the result of a direct action of the drug on the conceptus, an indirect action of the drug such as an alteration of a maternal or placental function which is essential to the well-being of the conceptus, or a direct or indirect action of a metabolite produced from the drug. The nature of the adverse effect may be death of the conceptus, malformation of an organ tissue or appendage, retardation of intra-uterine growth, or a functional or behavioral defect which may be detected at birth or may only become evident years after birth. Several papers have been published in recent literature reviewing the nature and mechanism of adverse effects of drugs on the conceptus (7-13).

A careful study of the literature leads one to believe that almost all drugs are capable of reaching the conceptus at all stages of gestation. During pre-implantation stages, the zygot or blastocyst is nourished by diffusion of nutrients into the cells from the fluid secreted into the lumens of the uterus and fallopian tubes (14-16). A wide variety of compounds and their metabolites are secreted into these fluids in significant quantities (17-19). The distribution of compounds into uterine and fallopian tube secretions, and their uptake from these fluids into the blastocyst, are influenced by such physicochemical factors as lipid solubility, degree of ionization, and molecular size of the compound (17-19). Molecules of a molecular weight of up to 19,000 have been shown to enter the rabbit

blastocyst, presumably by diffusion (18-19).

The placenta is formed after implantation. In humans, transfer across the placenta is the only significant route of material exchange between mother and fetus. However, water and electrolytes are exceptions, for these substances may be exchanged across the fetal membranes (amnion and chorion) between the amniotic fluid and uterine tissue (20). Studies of maternal to fetal transfer of drugs in humans indicate that any drug is capable of crossing the placenta if the maternal plasma concentration is sufficiently high for a sufficient period of time and if the drug is not metabolized by the placenta (5,6, 21,28).

The first consideration in choosing a drug for therapy in a pregnant woman should be to exclude those drugs known to affect the conceptus adversely. In cases where the effects on the conceptus are not known, the drug to which the conceptus will have the least exposure should be chosen. In the absence of information about placental transfer of a specific drug, the important question to be answered is: What are the factors which influence the rate and extent of transfer of the maternally administered drug from mother to conceptus? This work is an attempt to answer some aspects of this question as it relates to the human placenta at term.

Factors Affecting the Rate and Extent of Transfer Across the Mature Human Placenta

The mechanism of transport of various substances across biological membranes is generally attributed to one or a combination of the following: passive diffusion, facilitated

diffusion, active transfer, filtration, or pinocytosis (29). All of these mechanisms have been implicated in the transfer of various substances across the mature human placenta.

Passive Diffusion

This is a spontaneous process which occurs due to a difference in the chemical potential of a substance in various parts of a system, and which leads that system to a state of thermodynamic equilibrium. The rate of this process may be described by the following equation (30,31):

$$dq/dt = -(CDA/RT)du/dx \qquad [1]$$

in which;

dq/dt = the number of moles of the substance diffusing through a cross sectional area (A) at a point in the x direction per unit time,

C = the concentration of the substance at that point,

D = the diffusivity of the substance in the system,

R = the gas constant,

T = the absolute temperature, and

du/dx = the chemical potential gradient of the substance at point x.

The chemical potential gradient may be described as the sum of several gradients (36,32) as shown in equation 2:

$$du/dx = \bar{V}(dP/dx)-\bar{S}(dT/dx)-zF(dE/dx)+(RT/a)(da/dx) \qquad [2]$$

in which;

$\overline{V}$ = the molar volume of the substance,

dP/dx = the pressure gradient,

$\overline{S}$ = the molar entropy of the substance,

dT/dx = the temperature gradient,

z = the electrical charge of the substance,

F = Faraday's constant,

dE/dx = the electrical potential gradient,

a = the activity of the substance, and

da/dx = the activity gradient.

It is unlikely that a temperature gradient exists between the mother and fetus although in vivo temperature measurements have not been reported.

An electrical potential gradient between the mother and fetus has been shown to be absent in humans (33). This is also the case in rabbits (34). Nonzero maternal to fetal electrical potentials have been measured in goats (-71mv), sheep (-51mv) (35); guinea pigs (-18mv)(34); and rats (+15mv)(34).

A fetal to maternal hydrostatic pressure gradient may exist in vivo across the human placenta (36,37). In addition, a colloid osmotic pressure gradient has been measured in the maternal to fetal direction in humans (38). Aside from the contribution to the chemical potential gradient of all diffusible substances in the system, these pressure gradients would cause a net flow of water from fetus to mother. In turn, this net flow of water would cause all diffusible solutes to be carried by the process known as filtration or solvent drag. In such a case it has been shown that the contribution of the pressure gradient to the chemical potential term in the

diffusion equation is negligible compared with the mass transfer which occurs by filtration (39). The case of simultaneous transfer by filtration and diffusion will be considered in a subsequent section.

With the restrictions that $dP/dx = dT/dx = dE/dx = 0$, and with the realization that the concentrations at which most substances occur in the blood, is small so that activity (a) is approximately equal to concentration (C), the equation for passive diffusion of a substance at some point is reduced to:

$$dq/dt = - DA(dC/dx) \qquad [3]$$

Equation 3 is the familiar form of Fick's First Law of Diffusion (31).

If the placenta is visualized as a porous lipid membrane separating two well stirred pools of blood (maternal and fetal), and if the substance under consideration is neither accumulated nor metabolized in the membrane, then maternal to fetal diffusion may be considered to be the sum of two simultaneous processes: diffusion through aqueous channels or pores in the membrane, and diffusion through the lipid space of the membrane itself. Diffusion through the aqueous pores may be described by the expression:

$$dq/dt = - DAp(C_m - C_f)/T \qquad [4]$$

Maternal to fetal diffusion which takes place through the lipid space may be described by the expression (40):

$$dq/dT = - D_m A_m K(C_m - C_f)/T \qquad [5]$$

in which:

D = the diffusivity of the substance in the aqueous

channels,

D_m = the diffusivity of the substance in the membranes,

A_p = the effective pore area for substances diffusing through pores,

A_m = the effective area for substances diffusing across the membrane,

K = the partition coefficient for the substance between the membrane and maternal or fetal blood,

C_m = free (diffusible) concentration of the substance in maternal blood,

C_f = free (diffusible) concentration of the substance in fetal blood, and

T = the thickness of the membranes separating maternal and fetal blood.

The diffusivity, area, thickness, and partition coefficient terms are often combined for a particular substance and a particular membrane to define a constant called the diffusing capacity (Dc) (41) such that:

$$D_c = DA_p/T, \text{ for diffusion through pores,}$$

$$D_c = D_m A_m K/T, \text{ for diffusion through the membrane.}$$

In reality, the transport of most substances cannot be described by simply visualizing the placenta as a membrane separating two well-stirred pools of blood. A more realistic model is one in which transport is considered to take place between two flowing streams of blood separated by a porous lipid membrane. Thus, at any time, the concentration gradient is not the same at all points along the distance of the

membranes of exchange. The concentration gradient at any point along the diffusional path is a function of the concentration of the substance in maternal and fetal blood, the rate of blood flow in each stream, the arrangement and the direction of flow in each stream, the diffusing capacity of the membrane, accumulation of the substance in the membrane, and metabolism of the substance within the membrane. The rate of transfer of a substance across a membrane between two flowing streams can be described by the following equations which apply only under steady state conditions, i.e., $dC(x)/dt = 0$ at any point (x) in the system, and when there is no metabolism of the substance. It is also necessary to assume that the two streams flow in the same direction (concurrent flow), or in opposite directions (countercurrent flow) (30,41). Under these assumptions,

$$dq/dt = (C_{fa}-C_{ma})[(1/F)+(1/f)]^{-1}\{1-\varepsilon^{-Dc[1/F)+(1/f)]}\} \quad [6]$$

for concurrent flow; and

$$dq/dt = (C_{fa}-C_{ma})\{\{\varepsilon^{-Dc[(1/F)+(1/f)]}/F\} + (1/f)\}^{-1}\{1-\varepsilon^{-Dc[(1/F)+(1/f)]}\} \quad [7]$$

for countercurrent flow in which:

dq/dt = the amount of substance diffusing from maternal to fetal blood per unit time,

C_{fa} = concentration of the substance in fetal arterial blood,

C_{ma} = concentration of the substance in maternal arterial blood,

F = flow rate of maternal blood past the diffusing membranes,

f = flow rate of fetal blood past the diffusing membranes, and

ε = the base of the natural logarithm, i.e., 2.718....

These equations show that the rate of transfer is expected to be a function of the concentration gradient between fetal and maternal arterial blood, the diffusing capacity of the placenta, and the rates and relative directions of the fetal and maternal blood flows. For any particular condition of diffusing capacity, blood flows, and concentration gradient, exchange is more efficient when there is countercurrent flow. This greater efficiency can be seen by comparison of equations 6 and 7, and has been confirmed experimentally (42).

The vascular arrangement in the human placenta is such that the relationship between maternal and fetal blood flows is neither concurrent nor countercurrent. Maternal blood, from each arteriole opening into the intervillous space, flows past a series of fetal capillaries. The relationship of flows in the maternal stream to blood flow in individual fetal capillaries is a random mix of concurrent and countercurrent arrangements. This arrangement has been termed a "multivillous streambed system," and from equations developed to describe transfer of substances in such a system, its efficiency is found to lie between that of the concurrent and countercurrent systems (30,43).

If the diffusing capacity of a substance is quite small in numerical value of the blood flows (i.e., $D_c \ll F$ or f), equations 6 and 7, describing the steady state rate of transfer of a substance with either concurrent or countercurrent flow, can both be simplified to equation 8. Since the efficiency of the multivillous streambed system lies between that for concurrent and countercurrent systems, rate of transfer in such a system is also described by equation 8. Because transfer of such a substance is independent of the rates and relative direction of the blood flows, its diffusion is said to be permeability limited (41).

$$dq/dt = (C_{fa}-C_{ma})D_c \qquad [8]$$

On the other hand, when the diffusing capacity of a substance is large in numerical value compared to the values of the blood flows (i.e., $D_c \gg F$ or f), equations 6 and 7, and thus the equation describing rate of transfer in a multivillous streambed system, simplify to equation 9. Transfer of such a substance is termed flow rate limited diffusion (41) since its rate of steady state transfer is determined solely by the concentration gradient and the rate of blood flow.

$$dq/dt = (C_{fa}-C_{ma})[(1/F)+(1/f)]^{-1} \qquad [9]$$

From the theoretical considerations presented above, it can be seen that the factors which govern the rate of passive transport of substances across the placenta may be divided into three main categories: (1) the physico-chemical properties of the substance, (2) the properties of the placenta, and (3)

those factors influencing the concentration gradient across the placenta. These will now be discussed in turn.

Physico-chemical factors.--The physico-chemical properties of the substance will determine if the substance can cross the placenta through pores or lipid membranes of the cells, and aid in determining its diffusivity. Those properties which are important in this regard are molecular size, and oil-water partition coefficient at physiological pH. Molecular size will determine the ability of the molecule to pass through the pores of the membrane, and influences the diffusivity of the molecule which has an inverse dependence on molecular size (31). Because of the lipoid nature of plasma membranes, partitioning of substances into these membranes is similar to partitioning into oil or other nonpolar solvents, thus the greater the oil/water partition coefficient of the compound, the greater is its ability to partition into the plasma membrane and the more rapid is its diffusion across that membrane (29). For substances which partially ionize at physiological pH (weak acids and weak bases), the unionized form generally has a much higher oil/water partition coefficient, thus it is transferred more rapidly. The degree of ionization at a particular pH is dictated by the pKa (acid dissociation constant) of the compound or its conjugate acid, which thus aids in determining the oil/water partition coefficient of the compound at physiologic pH (29).

Placental factors.--Placental properties influencing the rate of passive diffusion of substances are: the surface area

available for exchange, the distance between the maternal and fetal blood streams, the nature of the barrier between maternal and fetal blood, and ability of the placenta to accumulate and metabolize substances as they cross, the rates and relationship of flow of the maternal and fetal blood streams. The effects of gestational age, pathological conditions, and drugs on the diffusional and metabolic properties of the membranes, and on placental blood flow must also be considered.

The total surface area of the fetal villi, with which maternal blood comes in contact, has been estimated to be 11-14 M^2 for the human placenta at term (44). Most villi show a profusion of microvilli which vastly increase this surface area (45). The total surface area of the fetal capillaries is probably a better estimate of the maximum surface area available for exchange between fetal and maternal blood. Recent estimates of fetal capillary area in normal pregnancy shows its value to average 74.8% of the villous surface at term (46).

The thickness of the tissues separating maternal and fetal blood in the human placenta at term has been estimated at 2-6 microns (44). This tissue is composed of several layers, which are, from maternal to fetal sides of the membrane: the syncytial trophoblast, cytotrophoblast, an epithelial basement membrane, connective tissue, and capillary endothelium. The ultrastructure of these layers will be summarized here as related to the placenta at term, and those properties which may influence transport.

Syncytial trophoblast has been described as being devoid of cell borders which would divide the cytoplasm and thus

provide an extracellular space through which maternal blood could make direct contact with fetal blood (45). More recent observers of placental syncytial ultrastructure have noted zones in which fibrin is adherent to the syncytium (47). Sections through these fibrin zones show they extend deep within the syncytium even to the basement membrane and may provide extracellular channels through the syncytial trophoblast. The thickness of the syncytium averages 10 microns but is reduced to 3-5 microns in areas of proximity to fetal capillaries.

The cytotrophoblast layer or Langhans cells, consists of distinct cells which rest directly on the epithelial basement membrane (45). In the term placenta, the Langhans cells are spaced widely apart allowing syncytial trophoblast to come between them and make direct contact with the basement membrane.

The epithelial basement membrane is a continuous layer separating the trophoblast layers from the connective tissue (45). Its thickness varies from 0.1 to 0.3 microns and tends to be thinnest where fetal capillaries lie close to the surface.

Between the basement membrane and the capillary endothelium is a layer of loosely arranged collagenous fibers which increase in abundance with gestation and which are occasionally anchored to the basement membrane (45). Among these fibers is a meshwork of fibroblasts and a dispersion of placental macrophages (Hofbauer cells). The thickness of the connective tissue layer may be as small as 0.2 micron.

The capillary endothelium is a single cell layer separating fetal blood from the connective tissue layer of the placenta (45).

Gross chemical analysis of the term human placenta has shown it to be composed of 85% water, 12% protein other than collagen, 1.3% collagen, 0.4% lipids, and 1.0% inorganic ash (48). The distribution of water within cells or in interstitial fluid is not known.

The high water concentration of the placenta should facilitate distribution of substances which accumulate throughout body water, while substances which accumulate in body fat would not be expected to accumulate in placental tissues.

Placental distribution of drugs should limit the rate and extent of transfer to the fetus, however, few studies have been made concerning accumulation of drugs in the human placenta. The results of investigations in which placental and fetal tissues were analyzed after administration of prilocaine to pregnant rats (49), saccharin to pregnant monkeys (50), and metronidazole to pregnant women (51) all show placental concentrations of the substances studied to exceed concentrations found in fetal tissue.

Several substances present on the surface of the syncytium or in intercellular spaces of the placenta may impede the transfer of some substances across the placenta by binding or by complexation. Fibrin, which is known to bind both anionic and cationic dyes (52) is prevalent on the syncytial surface and among intercellular material in the cytotrophoblastic layer (53). Albumin, which is present along the periphery of the fibrin deposits on the syncytium (53) is known to bind many substances of varied chemical structure (54). Mucin, which has been identified on the trophoblast surface (55) forms complexes with streptomycin and quaternary amines thus inhibiting

absorption of these substances across the gastrointestinal tract (56). The importance of these substances in influencing placental transfer of drugs has not been studied.

The ability of placental tissue to metabolize drugs in vitro has recently been reported by several investigators. The placenta has been found to be capable of metabolic transformations representing oxidation, reduction, conjugation, and hydrolysis of various drugs. Those drugs found to be metabolized by human placental tissue in vitro have been listed in a recent review on the subject (57). The significance of this in vitro capability to the in vivo situation has not been studied.

Maternal blood flow to the uterus at term is estimated at 500-600 ml/min of which 90% is thought to flow through the intervillous space of the placenta (58). Fetal blood flow through the placenta has been estimated by various investigators giving figures of 70 to 500 ml/min at term (59). The actual maternal and fetal flow rates of blood which is in contact with the membranes of diffusion may be less than these estimates since shunts may be present on either side of the membrane through which blood flows but does not come into contact with the membranes of diffusion.

Most of the factors considered thus far which influence passive transfer of substances across the placenta, change throughout gestation. The placenta attains its definitive architectural form by the end of the first trimester. The addition of new terminal villi continues, however, as gestation proceeds, increasing the total surface area exposed to maternal blood (58). The distance between the maternal and fetal blood

streams is reduced as gestation advances due to thinning of the cytotrophoblast layer and dilatation or looping of the fetal capillaries (46). The fetal capillary surface area increases from about 50% of the total villous area between 24 and 30 weeks gestation, to about 75% at term (46). Both maternal and fetal blood flow rates to the placenta increase throughout pregnancy (58). Deposits of fibrin on the syncytium become more prevalent with increasing gestation (53).

The net effect of these changes with respect to the placental permeability of the human placenta to sodium and water has been studied (60). In both cases placental permeability increases through the 36th week of gestation, after which permeability decreases. The decrease after the 36th week is thought to come about due to increased fibrin deposits.

A variety of pathological conditions may adversely affect placental transfer through an effect on blood flow, effective surface area, or permeability. The primary conditions affecting placental function are maternal malnutrition, impaired oxygen supply, circulatory disturbances, degenerative changes of or accumulation of abundant amounts of fibrin or calcium in the trophoblast, infections, and metabolic disorders (60).

Drugs through their actions on metabolic activity of the placenta, or alteration of blood supply to the placenta, may influence transfer. Effects of drugs on the placenta has recently been reviewed (57,60).

Factors affecting the concentration gradient.--Those factors which affect the concentration gradient of the drug between maternal and fetal arterial blood include the following:

the route and method of drug administration, the dose, protein binding of the drug in maternal and fetal blood, pH of maternal and fetal blood, and the disposition (distribution, metabolism, and excretion) of the drug in the mother and fetus.

The effect of dose can be illustrated by the fact that succinylcholine, a completely ionized drug which is rapidly hydrolyzed in the body, is not detectable in fetal blood after administration of usual clinical doses to the mother. However, if doses five to six times the usual clinical dose are administered, detectable concentrations of succinylcholine are found in fetal blood as soon as two minutes after administration to the mother (61). It is obvious that the greater the dose administered, the higher will be the concentration in maternal blood.

It is also obvious that the more rapidly a given dose of the drug is administered, the higher will be initial maternal blood levels of the drug and the more rapid the initial rates of placental transfer. The fact that higher levels of lidocaine are detected in the blood of infants whose mothers were given paracervical or pudenal blocks, than in the blood of infants whose mothers received the same dose of lidocaine by epidural injection can be explained by the more rapid absorption of lidocaine into maternal blood from the paracervical region (62).

The concentration of diffusible drug in maternal blood will be less than the actual concentration if the drug is bound to plasma proteins or maternal blood cells. Both these constituents of blood cross the placenta at rates which are extremely slow in comparison with the rates at which drugs

cross (63,64). This point is illustrated by the fact that the rate and extent of placental transfer of the highly plasma protein bound drug dicloxacillin was significantly less than the transfer of methicillin which is bound to plasma proteins to a lesser extent, even though identical doses of each drug were administered to pregnant women, and maternal plasma levels of dicloxacillin (the more lipid soluble drug) were about three times those of methicillin (65).

The extent and the rate at which administered drug is distributed throughout the body, and the rate at which drug is eliminated by metabolism and excretion are factors which determine concentration of drug in maternal arterial blood, the rate of change of concentration in maternal blood, and thus the rate of placental transfer (28,66). There have been few studies of drug distribution in pregnant women although numerous physiological changes take place which may potentially influence drug disposition in women during pregnancy. Among these changes are: hyperventilation, increases in plasma volume, whole blood volume, tissue fluid volume, cardiac output, renal blood flow, glomerular filtration rate; decreases in plasma protein concentration and hematocrit (5,67,69). Hepatic blood flow remains within normal limits during pregnancy in spite of the increase in cardiac output (70). The following alterations in drug disposition are expected due to the physiological changes described above: increased uptake of drugs administered by inhalation, increased rate and extent of distribution throughout the body, decreased rate of drug metabolism and/or excretion by the liver, increased renal clearance and increased elimination

of volatile substances from the lung. The fact that pregnant women are more rapidly induced and recover more rapidly from the effects of inhalation anesthetics is well known to anesthesiologists (5). Pregnant women have also been shown to have increased BSP retention (71-73), and to excrete unchanged a larger proportion of a dose of meperidine (74). These latter findings have been interpreted as showing a decrease in the drug metabolizing function of the liver during pregnancy, but may also be explained on the basis of changes in the ratio of liver to kidney clearance, and changes in volumes of distribution.

The concentration of drug in fetal arterial blood is a function of the amount of drug which has been transferred across the placenta, and the disposition of that drug by the fetus. The circulatory arrangement in the fetus is such that most of the blood returning from the placenta via the umbilical vein, perfuses the liver and then the brain, before perfusing the rest of the body (75). Distribution of substances which have crossed the placenta would be expected to be high into these two organs compared with distribution in adults and low in fetal lung which receives only 10-15% of the cardiac output (75). Distribution of substances in the fetal brain is enhanced further by the fact that this organ is deficient in myelin which forms the lipoid blood-brain barrier in adults (76). The fetal kidney does excrete drugs, but with an efficiency below that of adults, while the ability of the fetal liver to metabolize drugs is low if present at all (6,77). Significant quantities of some drugs appear in the amniotic fluid after drug administration to the mother (78,79). Transfer of drugs from the fetus into the

amniotic fluid takes place primarily via urinary excretion, and, to a lesser extent, by transfer across the fetal skin, mucosa, lungs, and the umbilical cord (80).

As is the case with diffusible drug in maternal blood, the concentration of diffusible drug in fetal blood will be reduced if the drug is partially bound by fetal plasma proteins. Results of binding studies of many drugs with fetal serum have shown that those drugs bound to maternal plasma proteins also bind to fetal plasma proteins but to a significantly lesser extent in each case (81-86). As a result, the extent of transfer to the fetus will not be as great as it would if the binding capacities were equal.

There is evidence that the difference in maternal and fetal plasma protein binding capacity for drugs is due both to: 1) significant binding of drugs by the plasma globulin fraction, the concentration of which is greater in maternal plasma; and 2) a binding capacity of maternal plasma albumin greater than that of fetal plasma albumin, the concentration of which is identical in both maternal and fetal plasma (82-87).

Measurements of fetal plasma pH have shown it to be 0.1-0.2 units below that of maternal plasma (88,89). This difference in pH might cause substances which are weak acids to partition toward the maternal side of the placenta, and substances which are weak bases to partition toward the fetal side, when the pKa of these substances is close to 7.2-7.4. This difference in pH is probably too small to be significant (90).

Facilitated Diffusion

The transfer of glucose across the placenta has been

found to be much more rapid than that of the closely related substances, xylose and fructose (91). In addition, net transfer always takes place in the direction of the concentration gradient. The mechanism of this transfer is known as facilitated diffusion and is thought to be a carrier mediated process.

Active Transfer

When the transfer of a substance takes place in the direction against the electrochemical gradient the process is known as active transfer. This process is also thought to be carrier mediated and outside energy must be provided. Natural amino acids and some vitamins are thought to cross the placenta by this process since their higher concentrations in fetal blood cannot otherwise be explained (92-96).

Filtration

If there is bulk movement of fluid through pores, aqueous channels, or breaks in the membrane as a result of pressure gradients, the transport of dissolved or suspended substances carried with the fluid is called filtration or solvent drag. As previously stated, there is evidence that hydrostatic and osmotic pressure gradients may exist across the human placenta *in vivo* and this would favor fetal to maternal flow of fluid (36-38). The significance of these findings to placental transfer *in vivo* is not known, but the filtration mechanism provides a possible explanation for the common occurrence of fetal blood cells in maternal blood, and the rare occurrence of maternal blood cells in fetal blood (22).

For the process of filtration, if passage of the solute

is restricted relative to the solvent and there is no concentration gradient across the membrane, the rate of fetal to maternal transfer of the solute may be described by the equation (39):

$$dq/dt = F_1 C_{fa} \qquad [10]$$

in which F_1 is the rate of fetal to maternal bulk fluid flow and can be described by the relationship:

$$F_1 = A_p(r)^2(\Delta P - \Delta \Pi)/(T8\eta)$$

in which r = the radius of the pores,

ΔP = the fetal to maternal hydrostatic pressure gradient,

$\Delta \Pi$ = the fetal to maternal osmotic pressure gradient,

T = the thickness of the membrane, and

η = the viscosity of the fluid (39).

If bulk flow of fluid is occurring while passage of the solute is restricted relative to the solvent, and/or there is a concentration gradient for the solute across the membrane, transfer will take place by the simultaneous processes of filtration and diffusion. In this event the rate of fetal to maternal transfer across a porous membrane separating two well stirred pools with fetal to maternal bulk flow of fluid can be described by equation 11 (39):

$$dq/dt = [D_c + (A_s/A_p)F_1]C_{fa} - D_c C_{ma} \qquad [11]$$

Pinocytosis

Pinocytosis is a process by which fluid and its constituents on one side of a membrane is engulfed by the

invagination and pinching off of a part of a membrane of that side. The vessel thus formed travels through the cytoplasm of the cell to the other side; there the process is reversed and the contents of this pinocytotic vessel are released outside the cell. What are suspected to be pinocytotic vessels have been observed in electromicrographs of placental villi (45,47). It is by this process that maternal plasma proteins are thought to gain access to the fetal blood stream.

Methods of Study

Often, placental transfer of a drug is inferred because effects which are typical of a drug, are observed in the neonate whose mother was administered the drug prior to delivery. Examples include reports of C.N.S. depression during the first week after delivery of an infant whose mother received 100 mg chlordiazepoxide daily during pregnancy (97), and prolonged hypoglycemia in the neonate whose mother was treated with chlorpropamide during pregnancy (98).

Direct proof of passage of a drug across placental membranes is provided by analysis of samples from fetal blood and other tissues.

Most placental transfer data in humans is collected by analysis of samples collected from the mother and fetus or neonate after a drug is administered to the mother prior to termination of pregnancy. A typical study of this type is one in which lidocaine was injected intravenously into pregnant women during labor. Immediately following delivery of the infant, samples of blood were drawn from a maternal artery, and from the umbilical vein and an umbilical artery of a doubly-

clamped segment of the umbilical cord (99).

Sometimes maternal blood is obtained from a vein (100), or directly from the intervillous space of the placenta by transabdominal aspiration (101). Often fetal blood is collected by simply allowing blood from the umbilical cord to flow into a test tube after the cord has been cut. This practice results in the collection of a mixture of blood containing uncertain proportions of umbilical venous and arterial blood. Blood from these two sources have quite different concentrations of the substance under investigation (28). Some studies also include sampling of amniotic fluid (65), recovery of the placenta for analysis (73), and/or sampling of fetal blood from scalp capillaries during labor (27). If termination of pregnancy is for therapeutic abortion, fetal tissues other than blood are often analyzed (102-104).

Collection of samples from the fetus prior to labor or termination of pregnancy has not been done in humans, however, collection of amniotic fluid, during mid-gestation in humans by amniocentesis has been done to study transfer of clomocycline (78).

There has been a report of an experimental procedure in humans which allowed collection of fetal samples from the same fetus at more than one time point (91). This procedure involved drawing blood at 5 minute intervals from the vein of the exposed umbilical cord of fetuses who were to be aborted subsequent to the experiment. However, for safety reasons, it is usually possible to obtain samples at only one time point for each fetus.

Experimental limitations are vastly reduced with the use of animals. Not only are more sampling procedures possible, but a wider variety of drugs may be used and any stage of gestation may be chosen for study. Caution must be observed in extrapolation of experimental results to humans since there are species differences in placental type, placental permeability, and disposition of drugs (105-107).

Small laboratory animals such as mice and rats may be employed in studies which result in termination of pregnancy. At a pre-determined time after injection of the drug, the animal is sacrificed and any or all maternal and fetal tissues may be collected and analyzed (108-110).

With the use of large laboratory animals such as monkeys, sheep, and goats, catheters may be implanted in maternal and fetal vessels and in the amniotic sac and multiple samples may be taken over a period of hours or days. This procedure makes possible kinetic study in the same animal, or performance of a series of experiments in the same animal at different times (41,50,111-114).

A technique involving surgical removal of the fetus and extracorporeal perfusion of the umbilical vessels of the placenta which is left attached to the uterus of the umbilical anesthetized mother has been performed in laboratory animals such as guinea pigs and rabbits as well as in humans (115-119). Maternal arterial and venous blood, as well as perfusate entering or leaving the umbilical vessels may be sampled. Drug either may be administered to the mother or may be introduced in the perfusate on the fetal side.

Transport of substances across the human placenta has been studied by perfusion ex vivo. Until recently, most studies have been performed by perfusing only the umbilical vessels while the maternal surface of the placenta is submerged in a stagnant or circulating bath (120,121). Krantz first reported the design of an apparatus intended to perfuse the intervillous space as well as the umbilical circuit (122,123). This apparatus simulates maternal circulation in the placenta by randomly puncturing the decidual surface of the placenta with numerous polyethylene tubes. Perfusate is pumped into the intervillous space through these tubes. Perfusate drains from the intervillous space by way of the venous openings in the decidua. The fetal vessels are simultaneously perfused by way of catheters inserted in the umbilical vessels. Various transport, metabolic, and physiologic studies of the placenta have been reported using this apparatus (123-135). Several other groups have reported placental perfusion devices which operate on the same principle as that of the Krantz apparatus (136-141).

Ex vivo perfusion of the fetal vessels and intervillous space of a single cotyledon of the human placenta has been reported by Panigel (142-145). This technique has the advantage of isolating one cotyledon which is in good condition from a placenta which may partially have visible damage.

Perfusion of the placenta ex vivo has the advantage of isolating placental factors from maternal and fetal disposition factors in the study of transport.

In vitro studies using placental tissues are not possible because of the difficulties in separating those

tissues involved in transfer. Permeability studies of the isolated amnion and chorion obtained from the fetal membranes attached to the placenta have been performed by mounting these membranes in a diffusion cell (146). The applicability of the findings of such studies to placental permeability *in vivo* is in question since the membranes studied may not be representative of the tissue layers limiting diffusion across the placenta. In addition, substances which show differences in permeability to fetal membranes *in vitro* may show no differences in their rates of *in vivo* transfer if transfer is blood flow rate limited (147).

Objective of the Study and Experimental Approaches to the Problem

The objective of this study was to investigate various factors which influence the distribution of drugs across the human placenta. It was of particular interest to test the hypothesis that drugs cross the placenta in relationship to their relative molecular sizes and their oil/water partition coefficients at physiologic pH.

It is generally assumed that the transfer of drugs across the human placenta takes place by passive diffusion, the rate of transfer being greater for smaller molecular weight and more lipid soluble drugs. Evidence for this assumption has been gathered mainly from *in vivo* experiments in which samples of blood taken from the mother and from the umbilical cord at the time of birth, are assayed for the drug, which is usually administered to the mother at some time during labor. However, in the *in vivo* experiment, it is not possible to separate the

effects of the physicochemical properties of the drug from the many factors previously discussed which affect the rate and extent of placental transfer (27,28). Thus, as several reviewers have stated (23-25,28), there is a need for a systematic study of placental transfer of substances differing in their physicochemical properties.

The ideal parameter to which correlation of oil/water partition coefficient and molecular size of various compounds should be made, is the diffusivity of the substance in the placental exchange membranes. However, the impracticality of isolating these membranes for an *in vitro* determination of diffusivity has been discussed in the previous section.

Diffusivity for a compound across the placental exchange membranes could be determined by an *in vivo* or an *ex vivo* perfusion experiment only if the rate of transfer of the substance and concentrations of the substance in maternal and fetal arterial blood are determined during steady state transfer of the substance, and if the surface area and thickness of the placental exchange membranes, the flow rates of maternal and fetal blood past the membranes of exchange, and the physical relationship of the maternal and fetal blood streams to each other are known. This can be confirmed by examination of equations 4-7. It should be noted, however, as demonstrated by equation 9 that the diffusivities of substances which are flow rate limited in their transfer across the placenta, cannot be determined by an *in vivo* or *ex vivo* perfusion experiment since the rate of transfer is only a function of the rates of maternal and fetal blood flow. In addition, it is difficult to determine

surface areas and thickness of membranes, flow rates past the exchange membranes (shunts may exist), and physical relationships of the blood streams to each other, in order to calculate diffusivities of substances which are not flow rate limited in their placental transfer.

The rate of transfer of a substance which is not metabolized by the placenta, and its concentration in maternal and fetal arterial blood, may, on the other hand, be easily determined during steady state transfer by an *in vivo* experiment, or by an *ex vivo* perfusion experiment. The ratio of the steady state rate of transfer for a substance (dq/dt) divided by the fetal-maternal arterial blood concentration gradient (C_{fa}-C_{ma}), as seen by rearrangement of equations 6-9, is a function of the diffusing capacity, the flow rates of maternal and fetal blood past the membranes of exchange, and the physical relationship of the maternal and fetal blood streams to each other. This ratio has been termed the diffusional clearance (Cl) of the substance (41).

If diffusional clearances for various substances are simultaneously determined in the same experiment, so that membrane parameters and flow rates are identical during the experiment, their comparison will be a measure of the relative diffusivities of the substances.

If one of the substances studied in such a comparative experiment is flow rate limited in its transfer across the placenta, as shown by equation 9, its diffusional clearance will be the maximum possible clearance (Cl_{max}) since its value depends only on the rates of maternal and fetal blood flow.

thus, diffusional clearances of substances whose diffusivities are sufficiently small so that transfer is not flow rate limited, will be less than Cl_{max} and the diffusional limitation (L_D) for the substance may be calculated for that substance by equation 12.

$$L_D = \frac{Cl_{max} - Cl}{Cl_{max}} \qquad [12]$$

The diffusional limitation of a substance may be defined as the relative limitation to the diffusional clearance of a substance due to its diffusivity (41,112).

Thus, although determination of diffusivities of various substances across placental exchange membranes can only be measured with great difficulty, if at all, the diffusional limitation of various substances, is a parameter which may more easily be determined. The magnitudes of the diffusional limitations will give rank order of the substances according to their diffusivities.

Therefore, if the hypothesis relating the physical properties of various substances and their rates of placental transfer is true, diffusional limitations determined as described above will increase with increasing molecular size and with decreasing oil/water partition coefficients.

The next step was to find an experimental model in which the rate of placental transfer and maternal and fetal arterial concentrations of various substances during steady state transfer could be determined and which would yield results relevant

to human *in vivo* placental transfer.

Obviously, pregnant women could not be employed for such experiments and the first system considered was *in situ* perfusion of the guinea pig placenta. The placenta of the guinea pig is histologically similar to that of the human (148) so it was felt the results would be qualitatively similar to results expected in humans. Money and Dancis (119) had described a method of perfusing the umbilical vessels of a guinea pig placenta left attached to the uterus of the anesthetized mother after surgical removal of the fetus. Using this method, the substances being studied could be added to a solution which is continuously infused into the jugular vein of the mother, or to the solution perfusing the fetal circuit of the placenta through the umbilical artery cannula. In either case, steady state for each substance may be achieved if perfusate leaving the fetal circuit of the placenta is not recirculated. Samples of maternal arterial blood may be obtained from the carotid artery for determination of the concentrations of the substances studied. Concentrations of substances in fetal arterial perfusate are calculated from the amount of each substance added to a known volume of perfusate. The steady state rate of transfer for each substance may be calculated as the product of the umbilical perfusion rate, and the difference in concentrations between the perfusate entering the fetal circuit of the placenta *via* the umbilical artery cannula and the perfusate leaving *via* the umbilical vein cannula which may be sampled at will.

Although this method seemed promising, attempts to implement it were frustrated by the lack of reliable commercial

sources of pregnant guinea pigs whose gestational ages were known, and by the tedious and often unsuccessful attempts to cannulate the tiny umbilical vessels.

Consideration for the use of the guinea pig *in situ* perfusion model was dropped when methods by which both maternal and fetal circuits of the human placenta could be perfused *ex vivo* came to our attention. Use of the human placenta would improve the relevancy of the data to human *in vivo* placental transport provided that the *ex vivo* perfusion was performed under physiological conditions close to those *in vivo*.

The method of perfusing a single cotyledon of the human placenta as described by Panigel (142) seemed especially promising. Establishment of steady state transfer by perfusion of maternal and fetal circuits of the placenta without recirculation of venous perfusates can be accomplished without the use of excessive volumes of perfusates since the flow rates required to perfuse only one cotyledon of the placenta are low.

Panigel's method as originally described (142) required the identification and cannulation of the remnant of a maternal spiral arteriole on the decidual surface of the placenta. Perfusate is pumped into the spiral arteriole to establish circulation in the maternal intervillous space. Perfusate drains from the intervillous space through the natural venous openings in the decidua. The fetal circulation is established by perfusing into the cannulated fetal arterial branch which enters the cotyledon in apposition to the cannulated spiral artery. Perfusate leaves the fetal circuit of the cotyledon *via* the vein which may be cannulated to collect the perfusate.

The substances to be studied may be added to either maternal or fetal perfusate, and since the compositions of the perfusates are known, only the perfusate leaving the fetal circuit *via* the vein needs to be sampled at steady state. The steady state rate of transfer is determined product of the fetal circuit perfusion rate times the arterio-venous concentration gradient on the fetal side.

The identification and cannulation of the maternal spiral arteriole remnant proved to be the greatest obstacle to the use of the Panigel method in this study. After numerous attempts, a spiral arteriole could not be located on the decidua of freshly delivered human placentas, or, when found, the spiral arteriole could not be cannulated as described by Panigel (142). It should be noted that more recent publications from Panigel's laboratory (143,145) indicate that his group no longer attempts to locate the spiral arteriole of placentas in order to establish the maternal circulation, but insert a glass cannula through the decidua in the center of the cotyledon.

The experimental model finally chosen for this study was *ex vivo* perfusion of both maternal and fetal circuits of the whole human placenta as described by Krantz, *et al*. (123). Criticism of this method has been voiced because the entire intervillous space of the placenta may not be perfused, and it is felt that damage to the villi may occur causing leakage of fetal perfusate from the fetal capillaries into the intervillous space (142). The first of these criticisms is not relevant to this study since the rank order of the diffusional limitations for the substances studied simultaneously will not be affected

by the actual rate of maternal perfusate flow past the placental exchange membranes, but only by the diffusivities of the substances. The bulk leakage of fetal perfusate to the maternal circuit will, however, cause serious problems in the experiment. The occurrence of this type of leakage may be monitored during the experiment by the addition of a substance to the fetal perfusate to which the intact fetal capillaries are impermeable. Appearance of this substance in the maternal circuit would be indicative of damage. A complete description of the apparatus used to perfuse the maternal and fetal circuits of the freshly delivered placenta and its operation can be found in the Materials and Methods section.

The fetal and maternal perfusion rates required to perfuse the entire placenta are sufficiently high as to make an open system perfusion, i.e., one in which perfusate leaving the circuits is not recirculated, impractical. Large volumes of perfusate are required to carry out the experiment for the length of time necessary to reach steady state. Steady state may be achieved, however, by the use of a "partially open" system perfusion. For the Krantz model, a partially open system may be designed in the following manner. Maternal and fetal perfusates, contained in separate reservoirs, are pumped into the respective arterial circuits while perfusate leaving maternal or fetal circuits is returned to its respective reservoir. In addition, perfusate is pumped at constant rates into both maternal and fetal reservoirs from separate external reservoirs. The well-stirred maternal and fetal reservoirs are kept at a constant volume by overflow tubes which allow the contents of

each reservoir to drain at rates equal to the rates at wh
perfusate is pumped from its external reservoir. The su
to be studied may be added to either the maternal or fet
reservoir and its external reservoir. After a period of
sufficient to reach steady state, transfer will take pla
across the placenta, away from the circuit to which the
stances are added, at a constant rate. The steady state
of transfer for each substance may easily be determined
measuring the rate at which each substance drains from t
reservoir opposite to which it is originally added, sinc
steady state the rate in is equal to the rate out. Conc
tions of the substances in perfusates entering maternal
arterial circuits may be determined by assay of perfusat
draining from the respective reservoirs.

After much preliminary experimentation with the
apparatus it became apparent that steady state experimen
not be carried out due to leakage from the fetal to mate
circuit of each placenta perfused. The leakage which oc
was not always due to physical damage to the placental v
but experiments in which a macromolecule was added to th
circuit indicated that water transfer was occurring even
there was no bulk leakage of fetal perfusate into the ma
circuit. Although this water transfer was no doubt due
fetal to maternal hydrostatic pressure gradient, attempt
prevent the loss of fluid from the fetal circuit by the
of a substance, whose purpose was to create an opposing
pressure gradient, were unsuccessful.

Rather than seek another experimental model in w

steady state experiments could be carried out according to the original experimental plan, it was decided to proceed with the use of the Krantz apparatus to study factors affecting placental transfer of various substances under non-steady state conditions. Of particular interest was the effect of placental uptake and the effect of fetal to maternal fluid flow on the rate and extent of placental transfer.

The experiments thus described are presented in separate sections.

The preliminary experiments first described were necessary in order to test the functional capability of the apparatus, and to collect data regarding the physiological performance of the ex vivo perfused placentas.

Once an appraisal was made regarding the functioning of the apparatus and placentas, experiments designed to study the comparative disposition of various substances using this experimental system were performed. Experimental observations included: 1) Comparison of initial disappearance rates from the circuits to which the substances were added; 2) Accumulation of the substances in the placenta; and 3) Appearance of the substances in the amniotic fluid chamber.

The substances chosen for study were antipyrine, sulfanilamide, N-4-acetyl-sulfanilamide, sufadimethoxine, sulfobromophthalein, blue dextran, and low molecular weight dextran.

Antipyrine (ANT) has a molecular weight of 188 and is known to cross body membranes rapidly and equilibrate with body water (149). The transfer of ANT across the human placenta is

known to be rapid (101) and its clearance across the placentas of sheep, goats, and monkeys has been shown to be flow rate limited (41,112,150-151).

Sulfobromophthalein (BSP) was originally included in the study for the purpose of monitoring leakage of fluid from the fetal circuit. However, preliminary studies with this compound showed that it was removed from the fetal circuit more rapidly than fluid and was detected in the maternal perfusate. This result was in conflict with the results of others who used BSP to monitor fluid loss from the fetal circuit during ex vivo perfusion of the human placenta (135,138). BSP was included with the compounds whose disposition in the ex vivo perfused human placenta was to be studied in order to further investigate this observation.

BSP has a molecular weight of 838 and is highly polar due to the presence of two sulfonic acid groups which are ionized at physiologic pH.

Blue dextran (BD) was chosen to monitor leakage from the fetal circuit after it was realized that BSP was not useful for that purpose. BD is a polymer of glucose having a molecular weight of 2,000,000. The BD molecule also possesses a covalently-bound blue dye. Because of its large size, BD would not be expected to leave the intact fetal circulation and its color facilitates visual and spectrophotometric detection.

Low molecular weight dextran (DEX) has an average molecular weight of 40,000. This compound was employed to provide colloid osmotic pressure to the fetal perfusate.

Sulfanilamide (SNL), N-4-acetyl-sulfanilamide (ASL), and

sulfadimethoxine (SDM), having molecular weights of 172, 214, and 310 respectively, are substances whose oil/water partition coefficients lie between those of ANT and BSP.

None of the substances described above were expected to exert any pharmacological effect on the placenta or to be metabolized by the placenta.

The initial disappearance rate of glucose (GLU) was also studied and compared with the initial disappearance rates of the drugs.

Because the experimental plan required the simultaneous addition of the substances in the perfusates, a major portion of the work dealt with the development of analytical methods to determine concentrations of all the substances present in the same sample.

MATERIALS AND METHODS

Drugs, Chemicals and Solutions

Drugs, Chemicals and Biologicals

Antipyrine (ANT)--Merck #1134

N-4-acetyl sulfanilamide (ASL) prepared from sulfanilamide and acetyl chloride by the method of Miller, et al. (152)

Sulfanilamide (SNL)--Eastman #4578

Sulfadimethoxine (SDM)--Hoffman La-Roche Laboratories

Sulfobromophthalein (BSP)--Sigma #S-0252 (disodium salt)

Anhydrous dextrose (GLU)--Merck #7263

Low molecular weight Dextran (average molecular weight = 40,000) (DEX) supplied either as a 10% solution--McGaw Laboratories or as Dextran-T-40^{R} powder--Pharmacia

Blue Dextran 2000^{R} (BD)--Pharmacia

Reduced nicotinamide adenine diphosphonucleotide (NADH)--Sigma #340-102

Lactate dehydrogenase suspension (LDH)--Sigma #826-6

Pyruvic Acid--Sigma #726-10

Nicotinamide-adenine-diphosphonucleotide (NAD)--Sigma #260-120

Lactic Acid standard solution 0.4 mg/ml--Sigma #826-10

All other chemicals used were of reagent grade or or better.

Ringer's Solution

NaCl	8.6 g
KCl	0.3 g
$CaCl_2 \cdot 2H_2O$	0.33 g
Distilled water, to make	1000 ml

Heparinized Ringer's Solution

To each liter of Ringer's Solution, add 5000 units of heparin.

Perfusion Solutions

The perfusion solution is prepared according to the following formula.

NaCl	5.44	g
KCl	0.298	g
$CaCl_2 \cdot 2H_2O$	0.147	g
$MgCl_2 \cdot 6H_2O$	0.203	g
Na_2SO_4, anhydrous	0.071	g
$NaH_2PO_4 \cdot H_2O$	0.138	g
$NaHCO_3$	2.27	g
Distilled water, to make	1000	ml

The electrolyte composition of this solution is shown below together with the electrolyte composition of human serum (153) for comparative purposes.

Ion	Perfusate Concentration (meq/l)	Serum Concentration (meq/l)
Na^+	123	142
K^+	4	4
Ca^{++}	2	5
Mg^{++}	2	2
Cl^-	101	101
SO_4^{--}	1	1
HPO_4^{--}	2	2
HCO_3^-	27	27
Organic acids	0	6
Protein	0	16

Perfusion solution is prepared in the morning if a placenta is expected during that day. The solutions are kept at

38°. If drugs, glucose, or dextran is to be added, they are added just prior to use along with 5000 units of heparin per liter of perfusate. The pH is then adjusted to 7.4 by the addition of a few drops of 1 N HCl. If perfusion solutions are not used the day they are prepared, they are stored in the refrigerator overnight, rewarmed the next morning, and discarded if not used the second day.

Amniotic Chamber Fluid

The solution used in the amniotic chamber is prepared as follows.

NaCl	5.84 g
KCl	0.298 g
$CaCl_2 \cdot 2H_2O$	0.257 g
$MgCl_2 \cdot 6H_2O$	0.203 g
$NaH_2PO_4 \cdot H_2O$	0.092 g
$NaHCO_3$	1.98 g
Distilled water, to make	1000 ml

The electrolyte composition of the amniotic chamber fluid is shown below together with the electrolyte composition of human amniotic fluid (153).

Ion	Amniotic Chamber Fluid Concentration (meq/l)	Human Amniotic Fluid Concentration (meq/l)
Na^+	126	135
K^+	4	4
Mg^{++}	2	1.4
Ca^{++}	3.5	3.6
Cl^-	109.5	109.0
HPO_4^{--}	2.0	0.7-1.2
HCO_3^-	24	unknown

The preparation and storage of this solution is identical to the procedure described for the perfusion solution. The pH is adjusted to 7.4 just prior to use by the addition of a few drops of 1 N HCl.

Equipment and Apparatus

Perfusion Apparatus

The perfusion apparatus used in these experiments was constructed based on the design of Krantz, et al. (119). Detailed plans and specifications for the construction of the perfusion apparatus may be found in their article. A schematic diagram of the apparatus is shown in figure 1.

The floor and walls of the amniotic chamber (ac) and the placental chamber (pc) are constructed entirely of clear plastic (Plexiglas^{R}). The beveled tips of 103 polyethylene tubes (pt) (ID 0.034", OD 0.060"--P.E. 100, Clay-Adams Div. Becton Dickenson) protrude 0.4 cm above the floor of the placental chamber. The other ends of the tubes are attached to a plastic manifold (mn) to which maternal perfusate (ma) is pumped from the reservoir.

Drainage of maternal venous perfusate (mv) from the placental chamber is accomplished by a collection groove (cg) located in the upper perimeter of the placental chamber which empties into four channels (dc) from which perfusate may be collected and returned to the reservoir. Rubber "O" rings (or) placed between the two sections of the manifold, between the placental chamber and the chamber spacer ring (csr), between the chamber spacer ring and the lower amniotic chamber cap (lac),

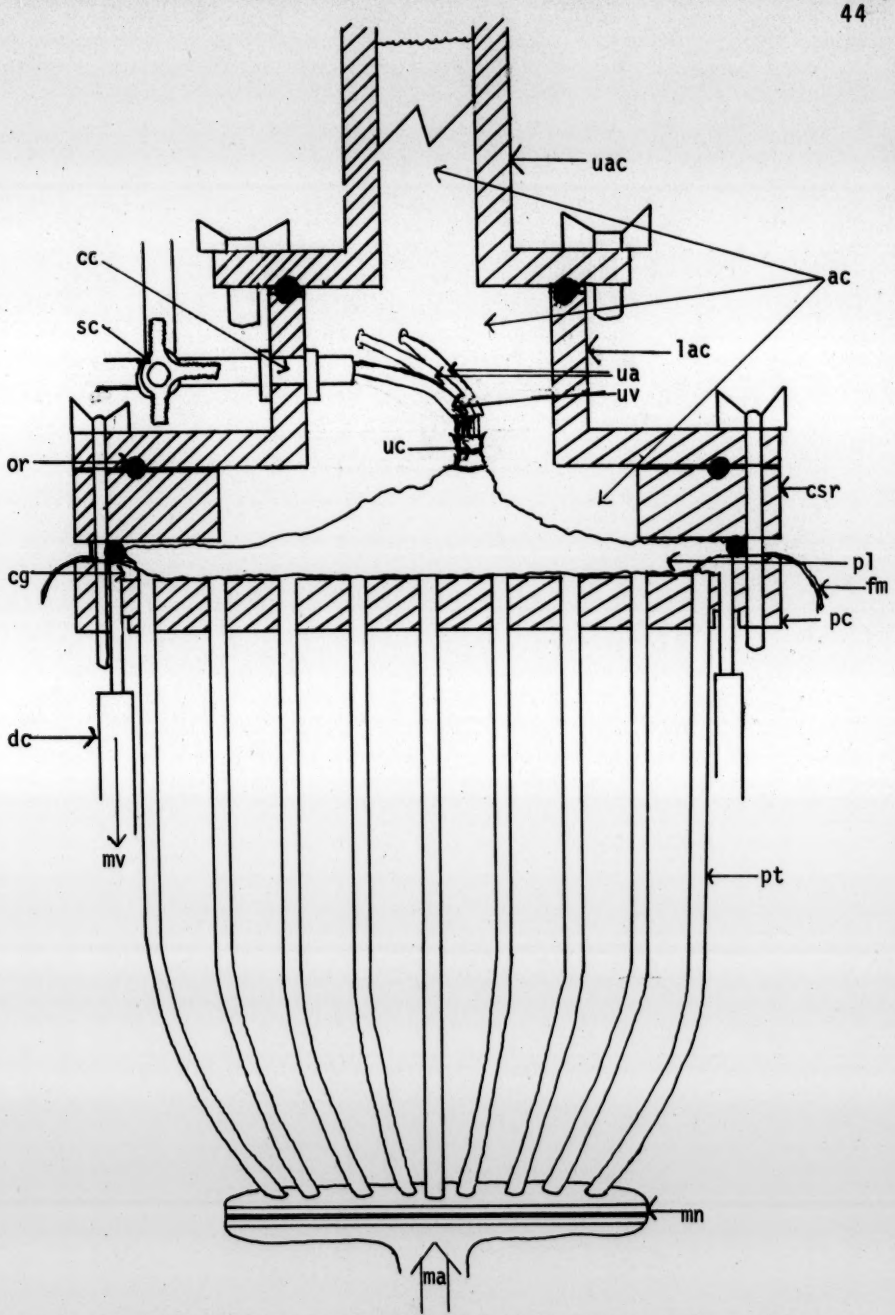


Figure 1. Schematic diagram of the placental perfusion apparatus showing a placenta (pl) with attached fetal membrane (fm) and umbilical cord (uc). See text for explanation of other abbreviations.

and between the lower amniotic chamber cap and the upper amniotic chamber cap (uac), provide watertight seals when the parts of the apparatus are clamped together.

Clear plastic tubing is sealed into holes drilled into the walls of the lower amniotic chamber cap to serve as connectors (cc) for tubing carrying fetal perfusate from the pumps and the umbilical artery cannulas (ua), and to connect the umbilical vein cannula (uv) with tubing returning fetal perfusate to the reservoir.

Stopcocks (sc), located between the cannula connectors and the tubing carrying fetal perfusate to or from the placenta, allow measurement of perfusion pressures without impeding perfusate flow.

Perfusion Pumps

A piston type pump with one-way ball valves (Pulsatile Blood Pump, Harvard Apparatus Co. #1405) is used to circulate maternal perfusate. Stroke rate and stroke volume are adjustable for this pump up to a maximum output of 1.6 liters per minute.

A tubing occlusion pump (Perastaltic Pump, Harvard Apparatus Co. #1201) is used to circulate fetal perfusate. The flow rate produced by this pump is dependent on the size tubing used and the gear setting.

Tubing

Silicon rubber tubing (Silastic, Dow Corning) is used in all perfusion circuits with the exceptions of the polyethylene tubing used in the perfusion apparatus, and Tygon tubing used in

the occlusion pump.

pH Meter

An expandable scale pH meter (Expandomatic, Beckman #76007) equipped with a combination pH electrode (Corning #476051) is used for all pH measurements.

Spectrophotometers

All ultraviolet and visible range spectra are measured on a Cary model 11 double beam recording spectrophotometer. A Beckman DB-G recording spectrophotometer is used to measure absorbance changes with time. All other absorbance readings are measured using a Spectronic-20 colorimeter, or a Beckman DU spectrophotometer.

Cannulas

A knotched, tapered, plastic tube, known as a Sim's tip obtainable from the Hospital Central Supply is used to cannulate the umbilical vein and both umbilical arteries.

Placentas

Placentas are obtained from the delivery rooms of the hospital adjacent to the building in which the perfusion laboratory is located. Only placentas from normal term uncomplicated pregnancies and deliveries are used.

Placental Perfusion Procedure

Procurement, Examination and Preparation of Placentas

Delivery room personnel are instructed to call when a patient expecting to have a normal-term delivery begins the

second stage of labor. At such time, about 500 ml of heparinized Ringer's solution, prewarmed to 38°C, is poured into an insulated container. The container and contents are weighed and taken to the delivery room area.

During delivery of the placenta, the umbilical cord is clamped to prevent drainage of fetal blood and subsequent collapse of fetal capillaries in the placenta.

Immediately upon delivery of the placenta a visual inspection is made to assure no lacerations or gross abnormalities are present. If the condition of the placenta appears satisfactory, the placenta is submerged in the warm heparinized Ringer's solution and rushed to the perfusion laboratory where the container and its contents are again weighed.

With one hand placed, palm down, over the fetal surface of the placenta, the entire container is turned upside down so that the placenta is held in the palm of one hand.

The diameter of the placenta is then measured to assure it is not too large to fit the placental chamber of the apparatus, the diameter of which is 20 cm, nor too small to completely cover the perfusion tubes, which occupy an inner circle of the placental chamber with a diameter of 16.5 cm.

The maternal surface is rinsed of blood with warm heparinized Ringer's solution, and the placenta is placed, maternal side down, in another container filled with fresh 38° heparinized Ringer's solution. During this whole procedure care is taken not to touch the maternal surface of the placenta.

The following information is recorded for each placenta: 1) wet weight of the placenta, 2) the longest dimension, 3)

shape (circular, oblong, irregular), 4) unusual features on maternal or fetal surfaces, 5) location of cord insertion (central, eccentric, etc.), and 6) apparent integrity of the membranes.

Cannulation of Umbilical Vessels

While the placenta is resting in the container of warm heparinized Ringer's solution, the umbilical cord is clamped with forceps about two inches above the placental insertion. The clamps are hung above the placenta so the cord is suspended in a vertical position.

The cord is then tightly clasped with the thumb and fore-finger of one hand, about one inch above its insertion. An incision is made through the cross section of the cord, just above the point where it is grasped by the fingers. The incision is made just deep enough so the three umbilical vessels are severed. An umbilical vein cannula, filled with warm heparinized Ringer's solution and stoppered at the distal end with a small glass rod, is gently inserted into the vein so the tip of the cannula just enters the point of placental insertion of the vein.

The umbilical vein is readily identified as the largest of the three vessels. To determine the integrity of the vessel, a tapered glass rod with a blunt tip is inserted into the lumen of the vein and gently eased downward to the insertion. Narrow tipped tweezers are used to expose the lumen of each umbilical artery. A procedure similar to that used to cannulate the umbilical vein is then carried out with each artery.

The cannulas are secured by making one or two ties around the whole cord with braided umbilical tape. Each artery is then gently flushed of blood with 100 ml of heparinized Ringer's solution delivered through a syringe attached to each artery cannula. The volume of blood flowing from the umbilical vein cannula is measured to detect the presence of gross leakage from fetal vessels. If no leakage is evident, the cannulas are then stoppered to prevent entrance of air into the arteries while awaiting connection to the perfusion system.

Mounting the Placenta in the Apparatus and the Initiation of the Perfusion

Both fetal and maternal arterial circuits of the perfusion apparatus are then primed with heparinized Ringer's solution warmed to 38°. The placenta is again placed in the palm of one hand and carefully lowered, maternal side down, onto the perfusion tubes in the placental chambers of the apparatus. During this step it is necessary to assure the placenta will be seated so that the umbilical cord insertion lies as close to the center of the chamber as is possible.

The fetal membranes are then spread over the sides of the placental chamber and temporarily held down by forceps clamped onto the edges of the membranes. In the event the membranes are not attached or are torn in such a manner that they would not adequately serve to separate the fluids of the placental and amniotic fluid chambers, a 12" x 12" latex rubber sheet is utilized for this purpose in the following manner.

A tiny hole is cut in the center of the rubber sheet. By stretching the sheet, the cannulated umbilical cord can be

forced through the hole. The sheet is pulled down over the cord to cover the fetal surface of the placenta. If the hole has not been cut too large, the sheet fits snugly against the umbilical cord and makes a watertight seal. The edges of the rubber sheet may then be spread over the sides of the placental chamber and clamped with forceps to hold the sheet tightly down. The chamber spacer ring and the lower amniotic chamber cap are set into place and clamped firmly to the placental chamber. The umbilical cord is then pulled up and the cannulas are attached to the cannula connectors on the inside of the lower amniotic chamber cap.

Perfusion of the fetal vessels is then started. At the same time artificial amniotic fluid is added through the opening in the top of the amniotic chamber cap. The hydrostatic pressure exerted on the placenta by the amniotic fluid forces the placenta down onto the beveled tips of the polyethylene tubes protruding through the floor of the chamber.

The maternal perfusion is then started. Next, the upper amniotic chamber cap is clamped in place and amniotic fluid is added to the desired level, through a small opening in the cap.

During this period, both maternal and fetal circuits are perfused with heparinized Ringer's solution which is supplied from a common reservoir.

The perfusate from each circuit is discarded until it is free of blood after which time the perfusate is recirculated.

After having ascertained satisfactory performance of the perfusion with respect to flows and pressures, and that both circulations are well washed of blood, the intake tubes for

both pumps are switched from the reservoir containing heparinized Ringer's solution to the maternal and fetal reservoirs which contain the perfusates whose composition are described in the experimental section. The venous outflows are also switched to the corresponding reservoirs. Figure 2 illustrates the relationships between the reservoirs and the maternal and fetal circuits of the placenta.

Flow Rate Measurements

Venous flow rates.--Floating ball type flowmeters (Gilmont) are placed in both maternal and fetal circuits between the venous outflow from the placenta and the reservoir. The flowmeters consist of a stainless steel ball enclosed in a tapered glass tube. When the flowmeter is held in a vertical position, the ball is suspended at a level which is dependent on the density, viscosity, and flow rate of the fluid which is passing through the tube. The level of the center of the floating ball is expressed in terms of a numbered scale etched onto the tube. Perfusion fluid warmed to 38° is pumped through the flowmeters at various known constant rates in order to calibrate the scale.

Arterial flow rates.--Fetal arterial flow rate is fixed for a particular size tubing and gear setting of the pump. The output of this pump in terms of flow rate is precalibrated with perfusion fluid at 38° for various tubing sizes and gear settings.

The maternal arterial flow rate is fixed by appropriate adjustments of the stroke volume and stroke rate of the piston pump.

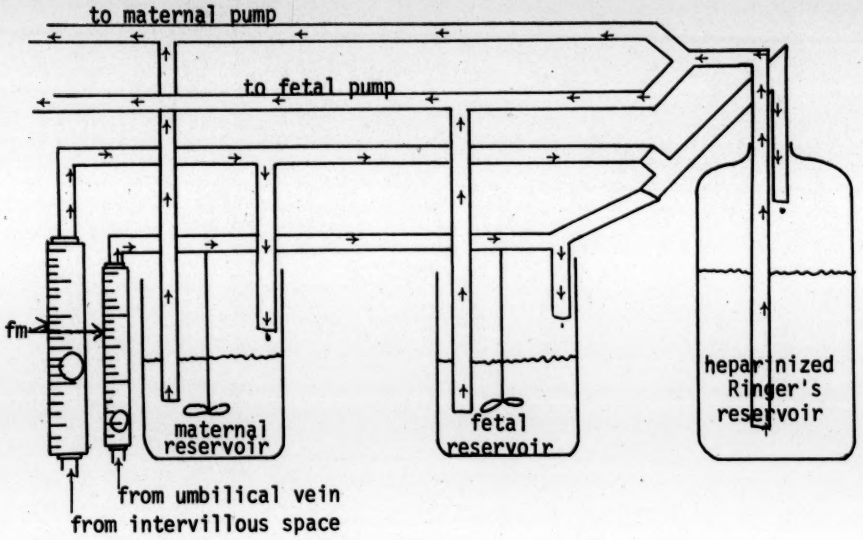


Figure 2. Schematic diagram of maternal and fetal perfusion circuits. Key: fm, flowmeters.

Pressure Measurements (figure 3)

Fetal arterial pressures.--A glass U-tube containing mercury is connected to the stopcock attached to each arterial cannula connector on the outside of the lower amniotic chamber cap. When the stopcock is opened to the U-tube, the pressure in mm Hg is read as the difference in mercury level between the two arms of the tube.

Fetal venous pressure.--Glass tubing is inserted into the stopcock attached to the venous cannula connector on the outside of the lower amniotic chamber cap. The tube is placed in a vertical position so that when the stopcock is open, the perfusate rises. The pressure exerted due to the weight of the perfusate in the tubing is equal to the venous pressure at that point. The error in assuming the perfusate density to be 1.0000 is calculated to be less than 2%. Therefore, the height of the column of perfusate in cm is read as the venous pressure at that point, in cm H_2O. Fetal venous pressure may be adjusted, with the use of a screw clamp, by alteration of tension on the tubing distal to the point of measurement.

Amniotic fluid pressure.--The height of the liquid in the amniotic fluid chamber is read directly as pressure in cm H_2O.

Intervillous space pressure.--One of the 103 polyethylene tubes which lead from the manifold to the placental chamber, is severed. A 20 gauge needle is inserted into the lumen of the severed polyethylene tubing which remains attached to the placental chamber. Silastic tubing is slipped over the hub of the needle and a glass tube is inserted into the other end of

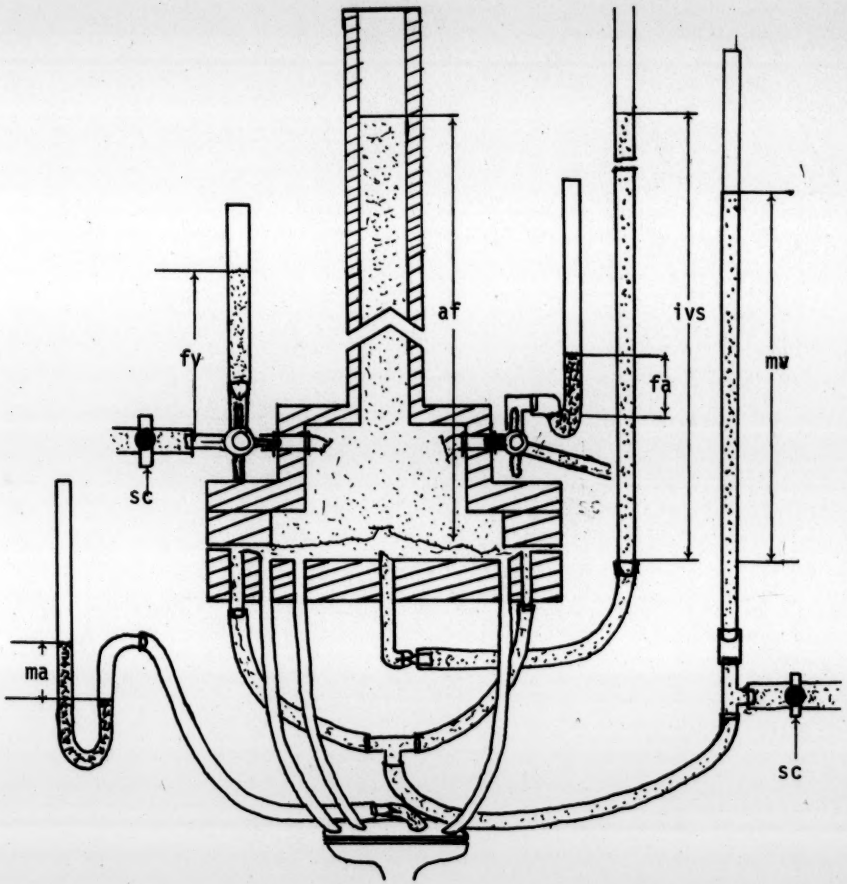


Figure 3. Schematic diagram illustrating the points of pressure measurements. Key: fa, fetal arterial pressure; fv, fetal venous pressure; ma, maternal arterial pressure; mv, maternal venous pressure; ivs, intervillous space pressure; af, amniotic fluid pressure; sc, screw clamp for adjusting venous pressures.

the tubing. When the glass tube is placed in a vertical position, the height of the fluid which rises above the level of the placental chamber is equal to the intervillous space pressure in cm H_2O.

Maternal arterial perfusion pressure.--Another 20 gauge needle is inserted into the severed end of the polyethylene tubing which remains attached to the manifold. Silastic tubing is used to connect the hub of the needle to a mercury filled glass U-tube. Pressure at this point in mm Hg can be measured as the difference in mercury level between the two arms of the U-tube.

Maternal venous pressure.--Glass tubing is placed in a vertical position and connected through a glass T-tube to the maternal venous circuit. The height of the fluid above the level of the floor of the placental chamber is the pressure at that point in cm H_2O. This pressure may be adjusted with the use of a screw clamp as described for fetal venous pressure.

Reservoir Volume Measurements (figure 4)

Reservoirs used for maternal and fetal perfusates are either one liter glass bottles calibrated in 50 ml increments between 200 and 1000 ml, or are clear plastic containers.

When the glass reservoirs are used, the reservoir volume is determined to the nearest 25 ml by reading the etched calibrations on the bottle.

In order to measure perfusate volume in the plastic reservoirs, a 10.0 ml pipet, calibrated in increments of 0.1 ml, is clamped in a vertical position on the outside of the water bath with silastic tubing connecting the lower tip of the pipet

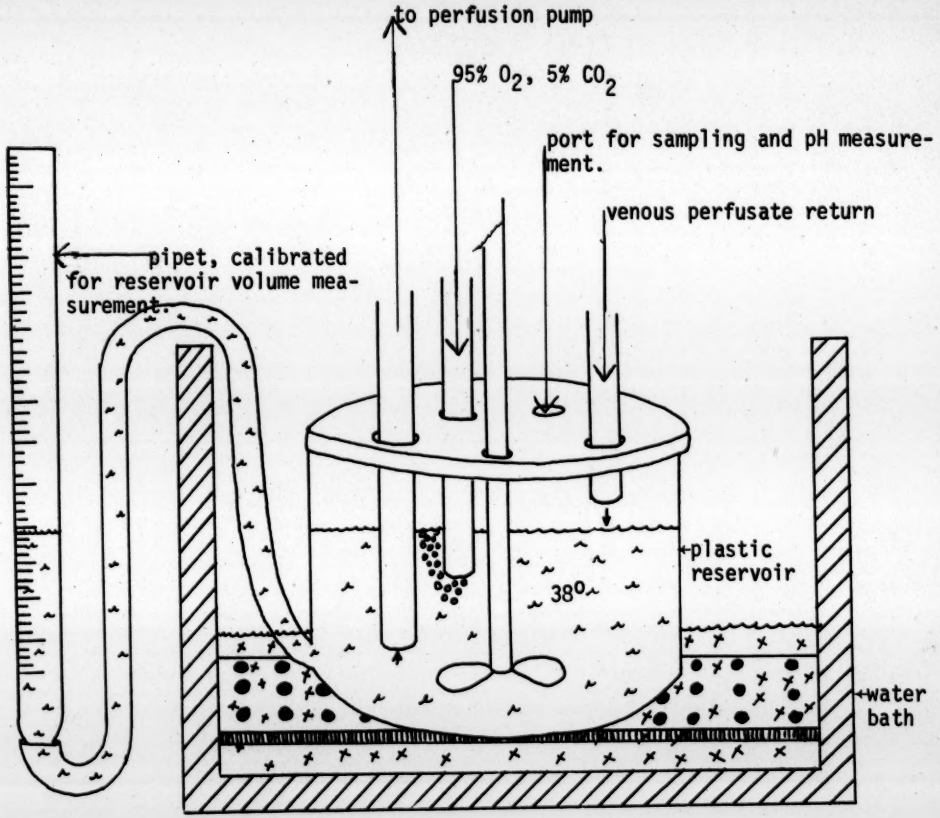


Figure 4. Schematic diagram of perfusate reservoir.

with a drain located near the bottom of the reservoir. When the pipet and tubing are primed with water, the level of the perfusate in the reservoir is identical to the fluid level in the pipet. The markings on the pipet are calibrated in terms of the volume of fluid in the reservoir by adding known amounts of perfusate to the reservoir and observing the pipet markings corresponding to the fluid level in the pipet.

Perfusate Temperature Measurement and Control

Temperature of perfusate in reservoirs is measured by insertion of a thermometer in the reservoir. Temperature of perfusate in the reservoir is maintained at 38° by adjustment of temperature of the water bath in which the reservoirs are immersed.

Oxygenation of Perfusate

Maternal perfusate is oxygenated by bubbling a mixture of 95% oxygen and 5% carbon dioxide into the maternal perfusate contained in the reservoir.

pH Measurements and Adjustments

The pH of perfusates is measured periodically during the experiment by inserting the electrode directly into the reservoir. When necessary, adjustment of perfusate pH is made during the perfusion by the dropwise addition of either IN NaOH or IN HCl directly into the reservoir.

Mixing of Reservoir Contents

Rapid mixing of reservoir contents is accomplished with plastic propellers constructed for that purpose which are

rotated with stirring motors suspended above the reservoir.

Sampling

An aliquot of each perfusate and of the amniotic fluid is taken at the start of each perfusion. Maternal or fetal arterial perfusate samples are pipetted directly from the appropriate reservoir.

Sampling of venous perfusates is accomplished by momentarily diverting venous perfusate into a small flask. The desired volume is pipetted from the flask and any excess perfusate is returned to the reservoir.

At the conclusion of each experiment, all the amniotic fluid is collected in a large beaker, mixed well, and an aliquot is taken for assay with the perfusate samples.

Samples are immediately centrifuged to remove any residual blood cells which may have been washed from the fetal vessels of the placenta or from the intervillous space during the perfusion. Those samples which are to be assayed for lactate and pyruvate are immediately deproteinated by the procedure described under assay procedures in order to prevent enzymatic conversion of pyruvate to lactate by any lactate dehydrogenase which may be present.

Assay Procedures

Deproteination of Samples

The following procedure adopted from the method of Marbach and Weil (154) is used to remove any proteins which may be leached from the placenta into the perfusate.

A 2.0 ml sample of perfusate is delivered directly into a cold centrifuge tube containing 6.0 ml of 5% metaphosphoric acid solution. The centrifuge tube is vigorously shaken for a few seconds and is set in an ice bath for at least ten minutes. The tube and its contents are centrifuged for at least ten minutes, removed from the centrifuge, and the supernatant is decanted into a test tube which is placed in a refrigerator until ready for assay. When assay of the protein residue is desired, the centrifuge tube is also refrigerated.

Calculations

Linear regression analysis by the method of least squares and solutions of simultaneous equations were performed using either a Hewlett-Packard model 9100A or a Cintra model 911 programmable desk calculator.

Blue Dextran (BD)

Aqueous solutions of BD show maximum absorbance in the visible range at 628 nm. Absorbance of samples containing BD or appropriate dilutions are measured at 625 nm.

As shown in figure 5, absorbance at 625 nm of BD in Ringer's solution shows a linear relationship with concentrations between 0.1 and 0.5 mg/ml. The absorbance of neutral or acidic solutions containing BD is unchanged in the presence of ANT and/or BSP.

Sulfobromophthalein (BSP)

Samples containing BSP are assayed according to the following procedures adopted from the procedure of Tindall and Beazley (71).

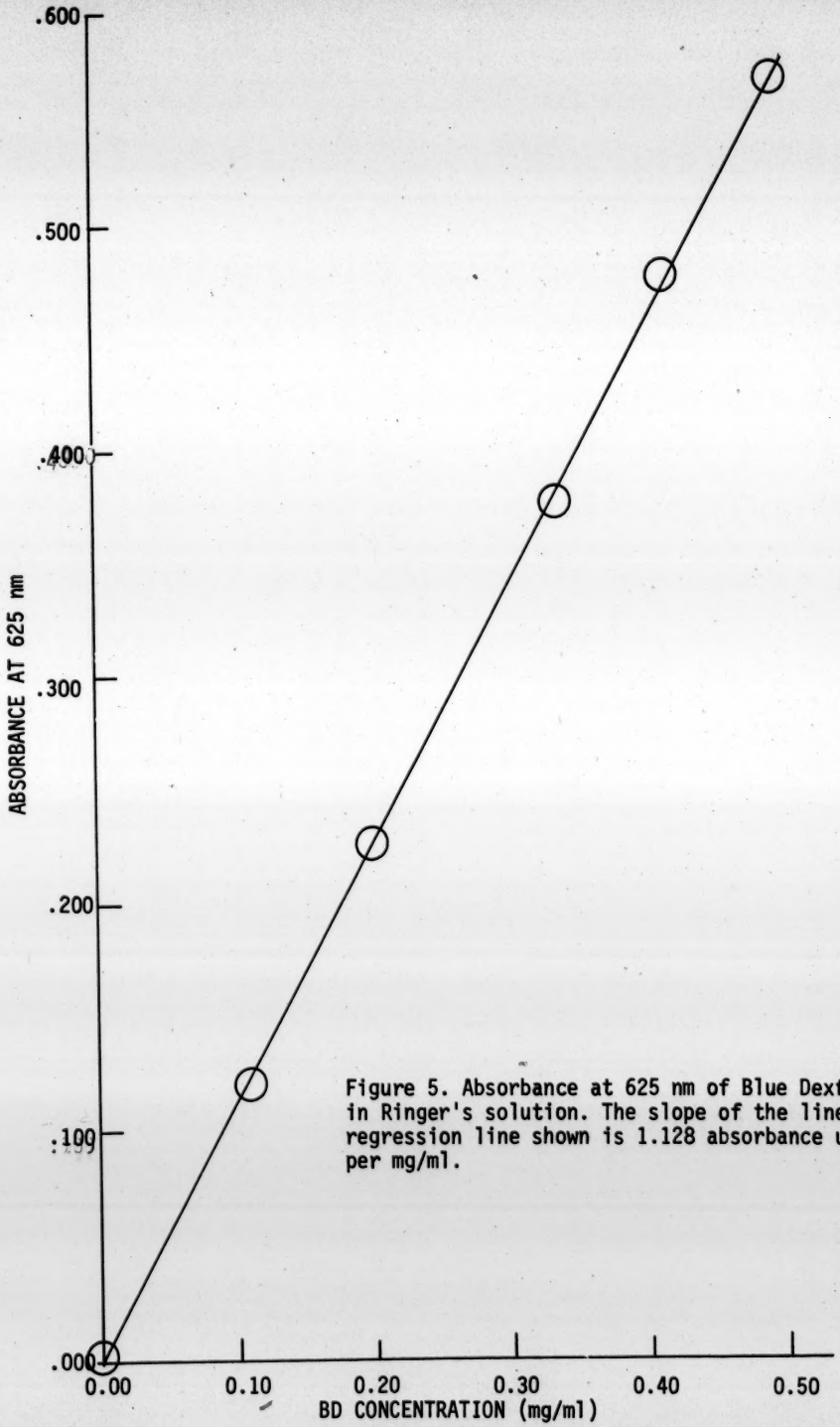


Figure 5. Absorbance at 625 nm of Blue Dextran in Ringer's solution. The slope of the linear regression line shown is 1.128 absorbance units per mg/ml.

Centrifuged samples.--A 1.0 ml aliquot of the centrifuged sample or a dilution is delivered into a graduated centrifuge tube. A solution of 0.5 N NH_4OH is added to the 15 ml mark and the contents are mixed well. The absorbance of the resulting solution is read at 580 nm.

The absorbance resulting from this procedure was linear with sample BSP concentrations ranging from 10 to 100 mcg/ml, and with sample BD concentration ranging from 1.2 to 5.5 mg/ml, figure 6.

If BSP and BD are present together in the same sample, the following procedure must be used in order to calculate BSP concentration. A 1.0 ml sample is pipetted into a test tube, 10.0 ml water is added and the absorbance read at 625 nm. After the addition of 4.0 ml of 1.75 N NH_4OH to the test tube the absorbance is read at 580 nm. The absorbance at 580 nm is corrected for the absorbance of BD subtracting the absorbance of the solution at 625 nm multiplied by the dilution factor (11/15) and by the ratio of the absorptivity of BD at 580 nm to its absorptivity at 625 nm. The concentration of BSP is calculated with the corrected absorbance by reference to the standard curve.

The absorbances of solutions of BSP or solutions containing BSP and BD were unchanged in the presence of ANT.

Deproteinated samples.--Partial precipitation of BSP occurs when the samples are deproteinated, therefore, the following procedure must be used.

Exactly 1.0 ml of the protein-free supernatant or an appropriate dilution is delivered into a test tube. A 5.0 ml

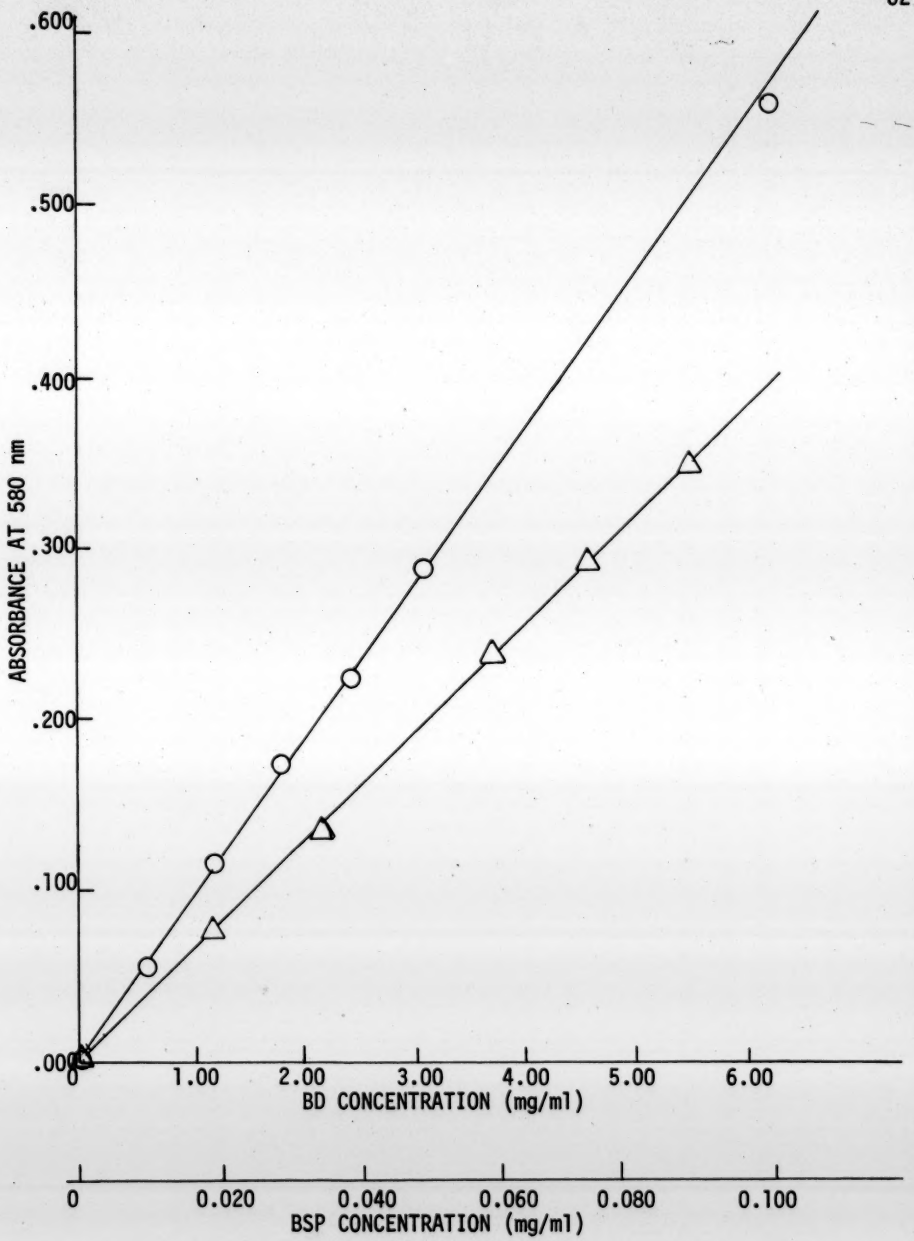


Figure 6. BSP assay procedure for centrifuged samples. Standard curves for BSP in Ringer's solution (○), and BD in Ringer's solution (△). Slopes of the linear regression lines are 5.58 a.u. per mg/ml BSP and 0.0631 a.u. per mg/ml BD.

quantity of 1N NH_4OH is added and the absorbance is read at 580 nm. The protein residue remaining in the centrifuge tube from the deproteinization is dissolved in enough 1N NH_4OH such that the color appears to be in the readable range. The absorbance of this solution is read at 580 nm. Concentrations of BSP for dilutions of both the supernatant and the residue are calculated by reference to a previously prepared standard curve and the amounts of BSP in each portion calculated.

The concentration of BSP in the original sample is calculated by dividing the total amount assayed by the 2.0 ml sample taken for deproteination.

Recoveries of BSP from perfusate by this procedure ranged from 96.3 to 101.1% (average 99.3) and recovery was not affected by the simultaneous presence of ANT, SNL, ASL, SDM, GLU, or DEX.

Antipyrine (ANT)

A modification of the method by Kuzel and Dolezal (155) is used for determination of ANT. Exactly 1.0 ml of the centrifuged or deproteinated sample is delivered into an eight inch test tube. A 2.0 ml volume of 36% acetic acid is added with mixing and the tube is placed in a water bath maintained at 25°. A 0.5 ml volume of 45% sodium nitrite solution is added with vigorous shaking and the tube is replaced in the water bath. Exactly 4 minutes after the addition of the sodium nitrite, 0.5 ml of 75% ammonium sulfamate solution is added and the tube is removed from the bath. When no more bubbles are seen to evolve from the solution, 2.0 ml of 7.5N H_2SO_4 is added, followed by 0.5 ml of 0.3% naphthoresorcinol in ethanol solution (NPR

solution). The absorbance of the resulting solution is read at 550 nm no sooner than 15 minutes, but within 24 hours after the addition of the NPR solution.

This assay is dependent on the formation of a colored compound having maximum absorption at 550 nm, which is formed by the condensation of naphthoresorcinol with 4-nitroso-antipyrine. The exact time and temperature of the nitrosation step is critical and 4 minutes at 25° is found to be optimum. The color develops slowly after the addition of the NPR solution and reaches a maximum absorbance after 15 minutes. The color is stable for 24 hours.

The absorbance of the condensation product is linear with respect to sample concentrations of ANT ranging from 5 to 25 mcg/ml, figure 7.

BD absorbs at 550 nm and absorbances of BD solutions on which the ANT assay is performed are linear with sample concentrations of BD ranging from 0.36 to 1.00 mg/ml, figure 7.

When BD is present in samples with ANT, a correction for the absorbance of BD at 550 nm is made in order to determine ANT concentration. This is done by a method similar to that used to correct for BD in BSP samples.

This procedure also forms colored compounds with SNL and SDM. Absorbances of the dyes produced from these compounds are identical on a molar basis and are linear with sample concentrations ranging from 0.01 to 0.40mM/l SNL or SDM. The absorptivity of the dye produced by the sulfonamides is 1.5% of the absorptivity of the dye produced by ANT at 550 nm on a molar basis, figure 8.

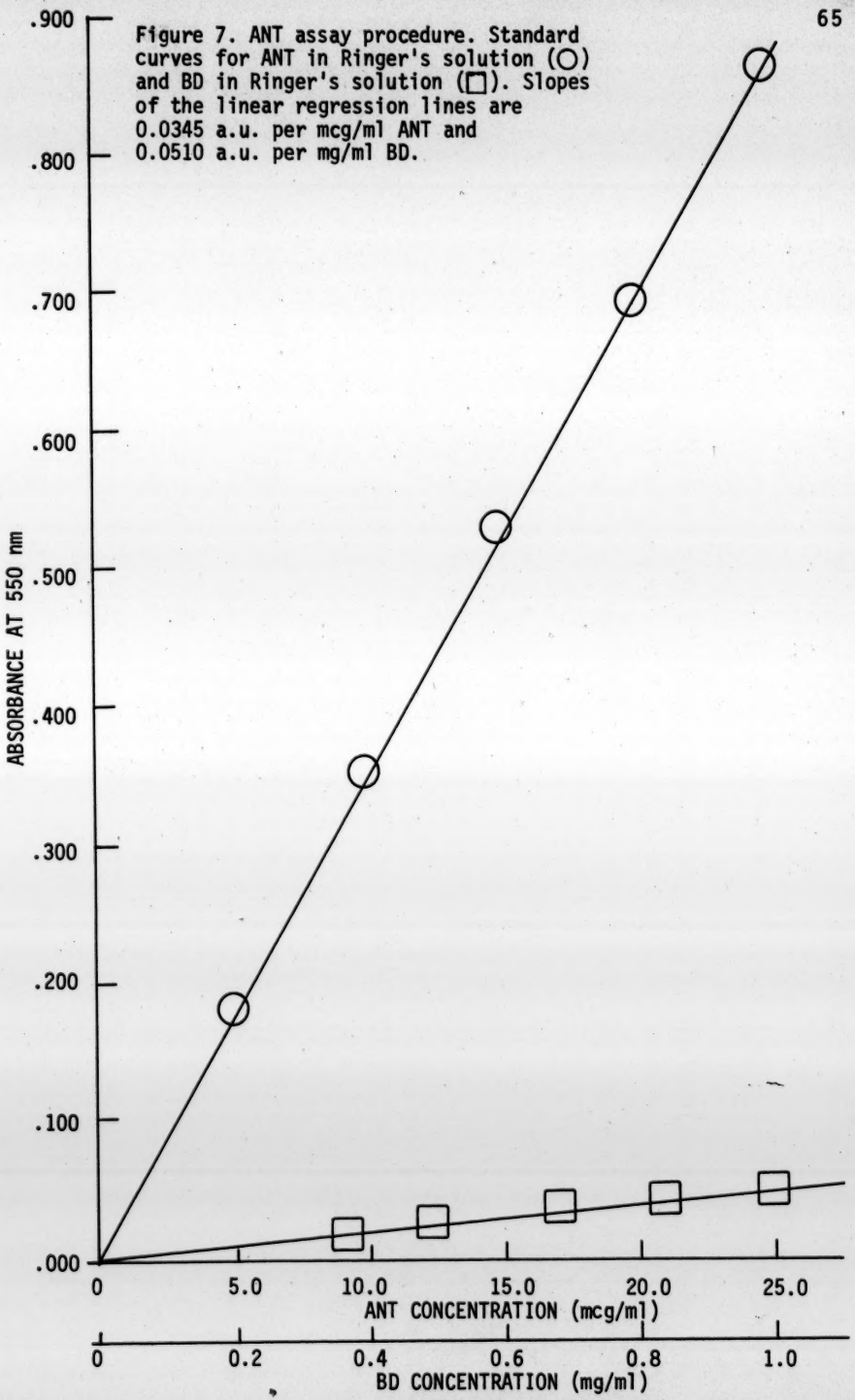


Figure 7. ANT assay procedure. Standard curves for ANT in Ringer's solution (○) and BD in Ringer's solution (□). Slopes of the linear regression lines are 0.0345 a.u. per mcg/ml ANT and 0.0510 a.u. per mg/ml BD.

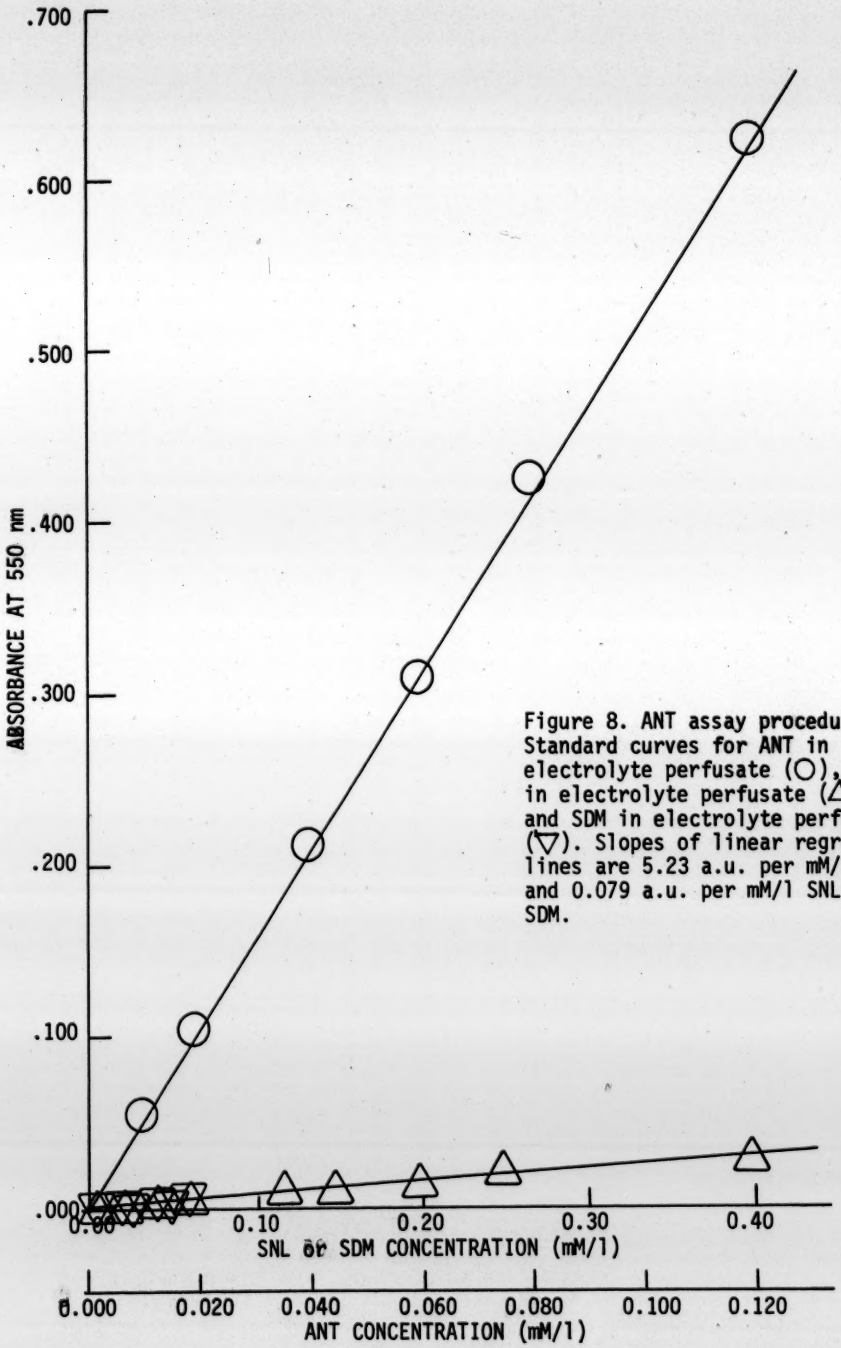


Figure 8. ANT assay procedure. Standard curves for ANT in electrolyte perfusate (○), SNL in electrolyte perfusate (△) and SDM in electrolyte perfusate (▽). Slopes of linear regression lines are 5.23 a.u. per mM/l ANT and 0.079 a.u. per mM/l SNL or SDM.

Simultaneous determination of ANT and SNL or SDM is discussed under the procedure for SNL or SDM.

There is no interference in this assay by the simultaneous presence of ASL, BSP, and/or DEX.

Dextrose (GLU)

Determination of GLU was carried out by the following method which is a modification of the method reported by Deckert (156).

A 0.5 ml sample of the deproteinated sample is added to a centrifuge tube followed by 4.0 ml of a freshly made solution containing 4.0 g p-bromoaniline in 100.0 ml thiourea-acetic acid reagent (10.0 g thiourea and 100.0 ml distilled water in enough glacial acetic acid to make one liter). The test tube is partially submerged in a 100° oil bath for exactly 10 minutes and is then allowed to cool to room temperature. The absorbance of the solution is read at 380 nm.

GLU solutions ranging in concentration from 2.0 to 12.0 mg/100 ml give absorbances by this procedure which are linear with sample concentrations, figure 9.

If DEX is present in the sample, a suspension of fine particles results during the heating step. The particles remain suspended and the tube must be centrifuged at 1700 RPM for at least twenty minutes. The clear solution which results will give absorbance readings at 380 nm which are higher than those given by samples containing no DEX. This additional absorbance is proportional to DEX concentrations in the sample ranging from 1.5 to 15.0 mg/ml as shown in figure 9. Simultaneous determination of GLU and DEX is discussed under the assay for DEX.

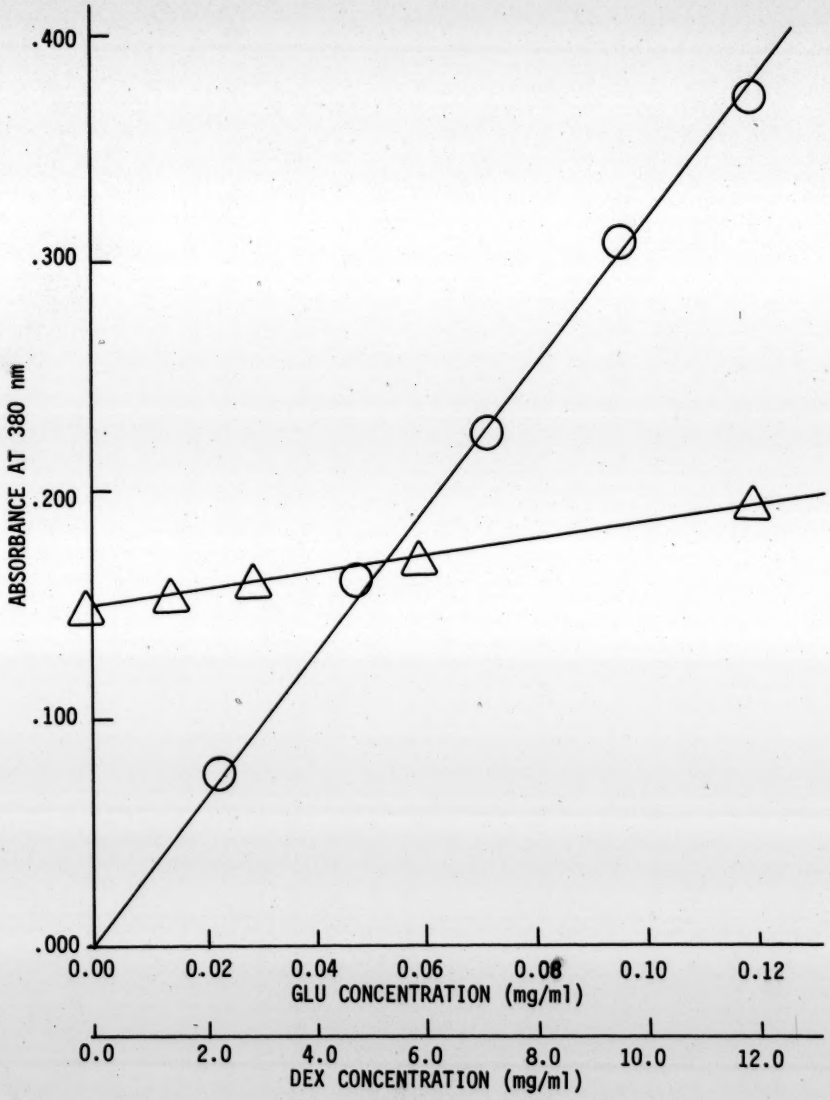


Figure 9. GLU assay procedure. Standard curves for GLU in electrolyte perfusate (○) and DEX in perfusate containing 0.045 mg/ml GLU (△). Slopes of linear regression lines are 3.18 a.u. per mg/ml GLU and 0.00372 a.u. per mg/ml DEX.

The simultaneous presence of ANT, SNL, SDM, ASL, and/or BSP will not interfere with this assay.

Pyruvate

Determination of pyruvate is based on measuring the amount of a cofactor, NADH, which is oxidized during the enzymatic reduction of pyruvate (154,157,158). Tris buffer, 0.75 M is freshly prepared by dissolving 9.08 g trishydroxymethlaminomethane in enough double distilled water to make 100.0 ml. NADH solution is prepared by adding 4.3 ml of the freshly prepared tris buffer to a vial containing 2.0 mg NADH. A 2.0 ml portion of the deproteinated sample is transferred to a test tube and is followed by addition of 0.5 ml tris buffer and 0.5 ml NADH solution. Absorbance of this mixture is read at 340 nm. To the mixture is then added 0.050 ml LDH and the absorbance is again read at 340 nm between ten and twenty minutes after addition of the LDH. The change in absorbance is calculated by subtracting the second reading from the first.

The change in absorbance at 340 nm is proportional to sample concentrations of pyruvate ranging from 0.02 to 0.12 mM/l, figure 10.

No interference is caused by the simultaneous presence of GLU, DEX, or lactic acid. Pyruvate cannot be determined in the presence of BSP since BSP absorbs at the pH in which this procedure is carried out.

Lactate

Determination of lactate is based on measuring the amount of NADH which is produced during enzymatic oxidation of

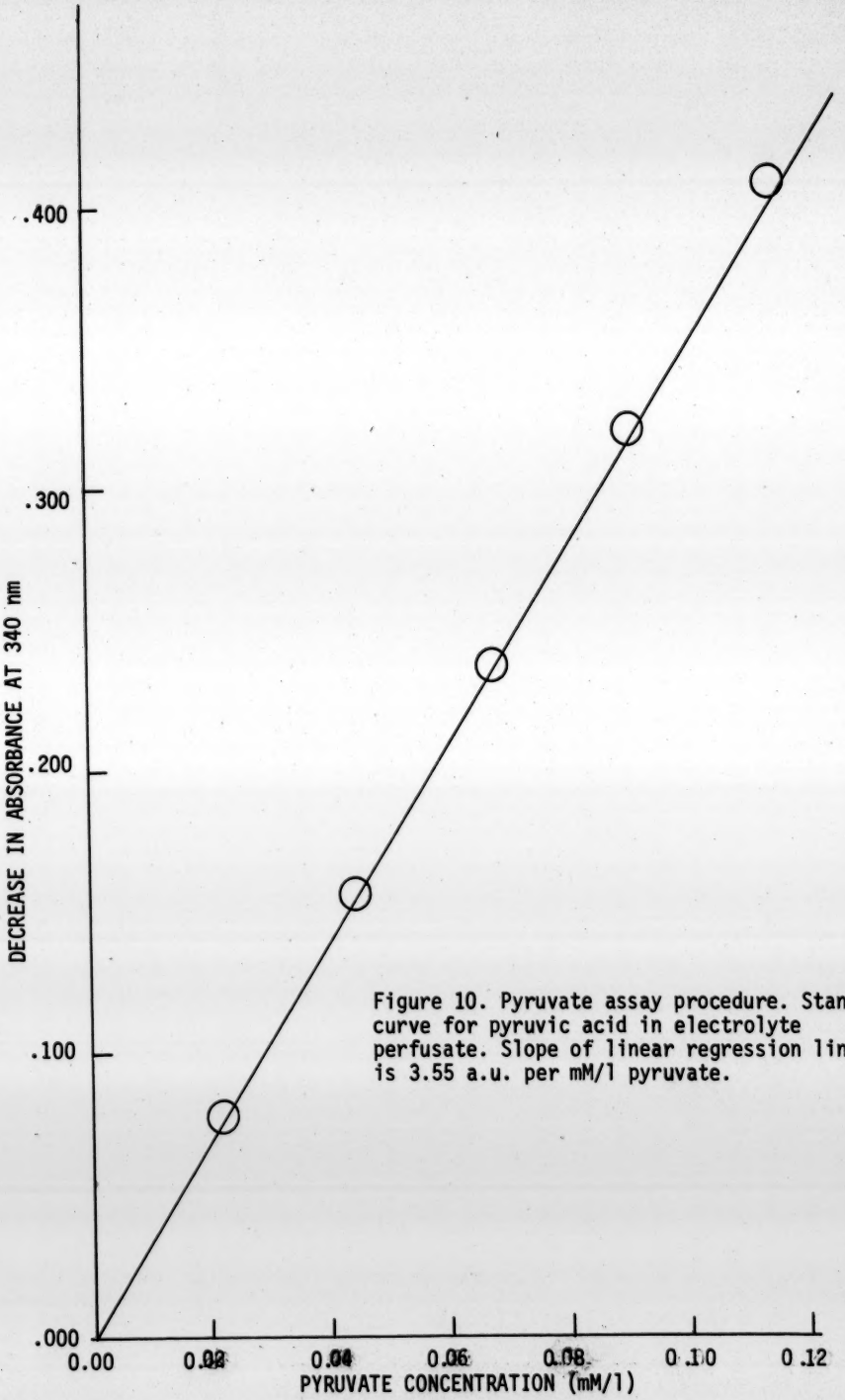


Figure 10. Pyruvate assay procedure. Standard curve for pyruvic acid in electrolyte perfusate. Slope of linear regression line is 3.55 a.u. per mM/l pyruvate.

lactate in the presence of NAD (154,158,159). A buffer (GH buffer) is prepared by dissolving 3.75 g glycine and 0.1 g disodium ethylenediamine tetra-acetic acid in a little water. Next, 1.0 ml hydrazine hydrate, 10.0 ml 2 N NaOH, and enough double distilled water are added to make 50.0 ml. The pH is adjusted to 9.5 with NaOH if necessary. NAD solution is prepared by dissolving 0.050 g NAD in 25.0 ml volumes each of double distilled water and GH buffer. An aliquot of the NAD solution is placed in the reference cuvette of the spectrophotometer. A 0.1 or 0.2 ml portion of the deproteinated sample is added to 2.0 ml NAD solution in the sample cuvette and the absorbance is read at 340 nm. A 0.050 ml volume of LDH is added with stirring to the contents of the sample cuvette and a continuous recording is made of the absorbance at 340 nm. The maximum absorbance is determined from the recording of sample absorbance vs. time and the difference between the maximum absorbance achieved after addition of LDH and the absorbance before LDH addition is calculated.

The maximum increase in absorbance is linear to concentrations of lactic acid in the final reaction solution ranging from 0.5 to 3.75 mcg/ml, figure 11.

No interference is caused by the simultaneous presence of GLU, DEX, or pyruvic acid. Lactate cannot be determined in the presence of BSP since BSP absorbs at the pH in which this procedure is carried out.

Dextran (DEX)

Determination of DEX is based on the method of Jensen, et al. (160). A 2.0 ml aliquot of an appropriate dilution of

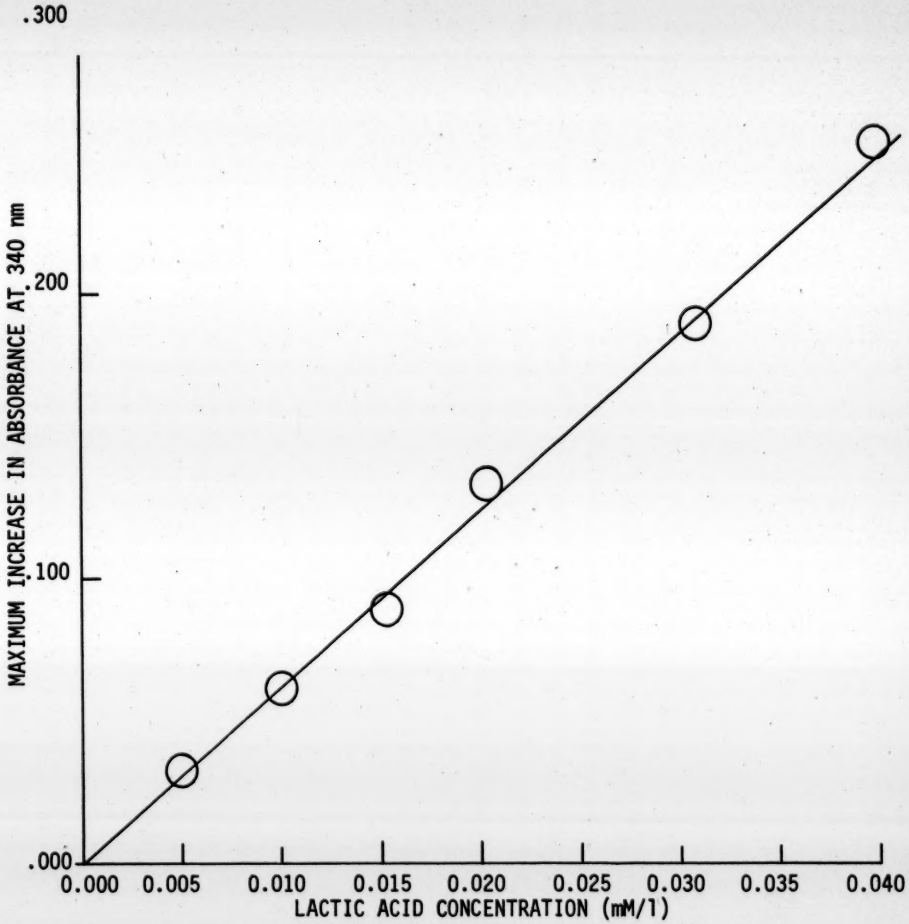


Figure 11. Lactate assay procedure. Standard curve for lactic acid in electrolyte perfusate. Slope of linear regression line is 6.15 a.u. per mM/l lactate in the final reaction mixture.

the deproteinated sample is pipeted into a large test tube and 3.0 ml of a reagent containing 0.2% anthrone in concentrated sulfuric acid is added. The tube is immediately placed in an oil bath maintained at 100°. The tube is removed from the bath after exactly eight minutes and is rapidly cooled to room temperature under a stream of cold water. The absorbance of the solution is read at 625 nm within five minutes of removal of the tube from the bath.

Because this method depends on the production of a colored complex of anthrone with the glucose units of DEX, GLU present in the sample also reacts.

Absorbances produced by solutions of GLU or DEX were linear with sample concentrations ranging from 5 to 20 mcg/ml GLU, figure 12.

Simultaneous determination of GLU and DEX concentrations is accomplished by performing both GLU and DEX assays on the sample and solving the following simultaneous equations for (DEX) and (GLU).

$$A_{625} \times DF_{DEX} = 123.2 \times (GLU) + 137.3 \times (DEX)$$

$$A_{380} \times DF_{GLU} = 31.8 \times (GLU) + 0.0372 \times (DEX)$$

in which:

A_{625} = the absorbance reading at 625 nm of the solution resulting from performing the DEX assay on the diluted sample;

DF_{DEX} = the appropriate dilution factor used for the sample in order to perform the DEX assay;

A_{380} = the absorbance reading at 380 nm of the solution resulting from performing the GLU assay on the diluted sample;

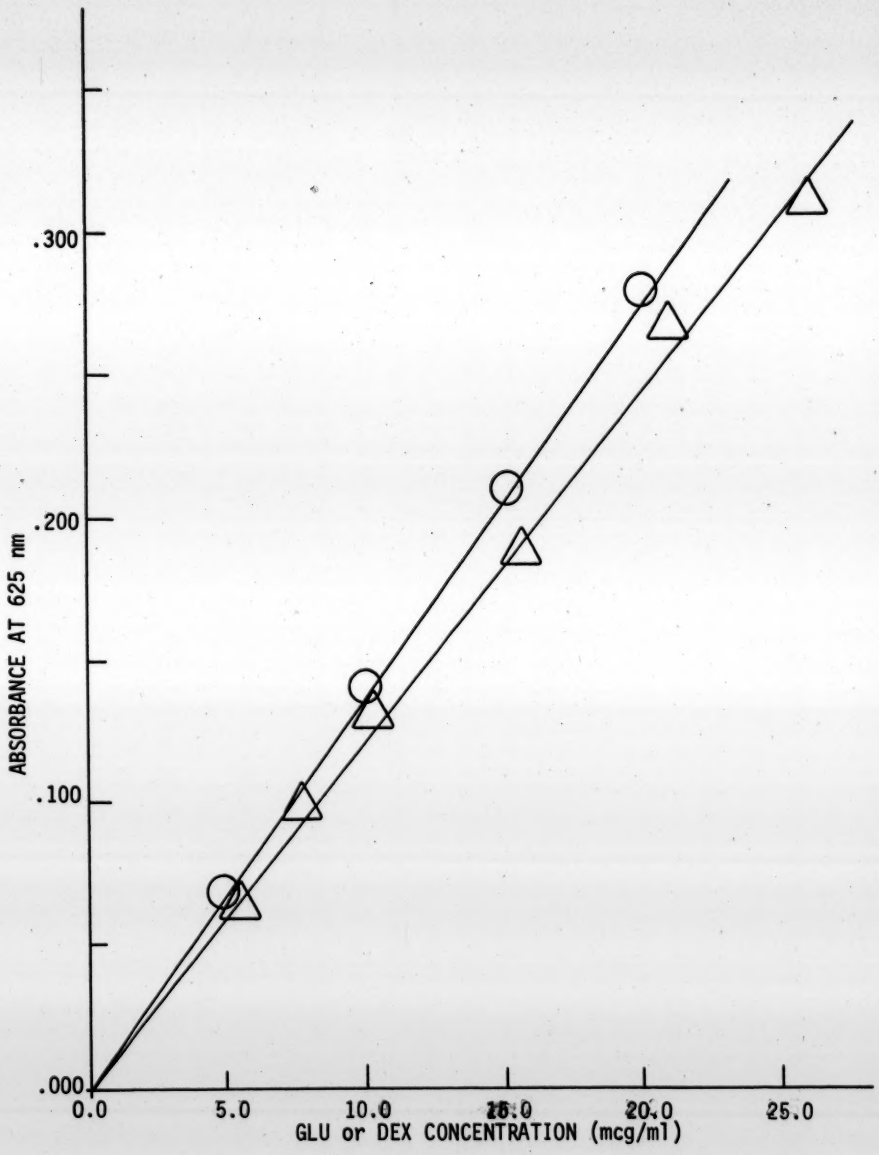


Figure 12. DEX assay procedure. Standard curves for DEX in electrolyte perfusate (○) and GLU in electrolyte perfusate (△). Slopes of linear regression lines are 0.01373 a.u. per mcg/ml DEX and 0.01232 a.u. per mcg/ml GLU.

DF_{GLU} = the appropriate dilution factor used for the sample in order to perform the GLU assay;

(GLU) = concentration of GLU in g/100 ml;

(DEX) = concentration of DEX in g/100 ml; and

the numerical coefficients of (GLU) and (DEX) represent the slopes of the standard curves for each assay calculated by linear regression analysis.

Interference is not produced by the simultaneous presence of ANT, SNL, ASL, BSP, lactic acid, and/or pyruvic acid.

Sulfanilamide (SNL) or Sulfadimethoxine (SDM)

Determination of SNL or SDM is performed by a modification of the procedure of Bratton and Marshall (161). The principle of this procedure is the diazotization and coupling of free aromatic amino groups with a compound to produce a dye.

A 1.0 ml aliquot of the sample is placed in a test tube followed by 0.5 ml 0.8 N HCl and 0.5 ml 0.4% sodium nitrite solution. After two minutes, 0.5 ml 2% sulfamic acid solution is added with vigorous shaking. After an additional two minutes, 0.5 ml 0.4% N-1, naphthylethylenediamine dihydrochloride solution (BM reagent) is added, followed by 5.0 ml 0.1 N HCl. Additional 0.1 N HCl may be added in 4.0 ml increments if necessary to adjust the absorbance to below 0.600. The absorbance is read at 540 nm exactly two minutes after addition of the BM reagent.

ANT also produces a colored compound when subjected to this procedure. Absorbances of solutions containing SNL, SDM, or ANT are linear with sample concentrations ranging from 0.01

to 0.10 mM/l SDM or SNL, and from 0.04 to 1.20 mM/l ANT, figure 13.

The simultaneous presence of ASL, BSP, GLU, and/or DEX causes no interference with this procedure.

The determination of ANT and SNL or SDM concentrations when both are present in the same sample is accomplished by performing assays for ANT and for SNL or SDM and solving the following simultaneous equations for mM/l ANT and mM/l SNL or SDM.

$$A_{540} \times DF_{SUL} = 6.56 \times \text{mM/l SNL or SDM} + 0.0375 \times \text{mM/l ANT}$$

$$A_{550} \times DF_{ANT} = 0.079 \times \text{mM/l SNL or SDM} + 5.23 \times \text{mM/l ANT}$$

in which:

A_{540} = the absorbance reading at 540 nm resulting from performing the SNL or SDM assay on the diluted sample;

DF_{SUL} = the appropriate dilution factor used for the sample to perform the SNL or SDM assay;

A_{550} = the absorbance reading at 550 nm resulting from performing the ANT assay on the diluted sample;

DF_{ANT} = the appropriate dilution factor used for the sample to perform the ANT assay;

and the numerical coefficients of mM/l SNL or SDM and of mM/l ANT are the slopes of the standard curves for each substance in the assay calculated by linear regression analysis.

The linear regression coefficients calculated for SNL or for SDM in each of the assays did not differ significantly and data from both standard curves were pooled.

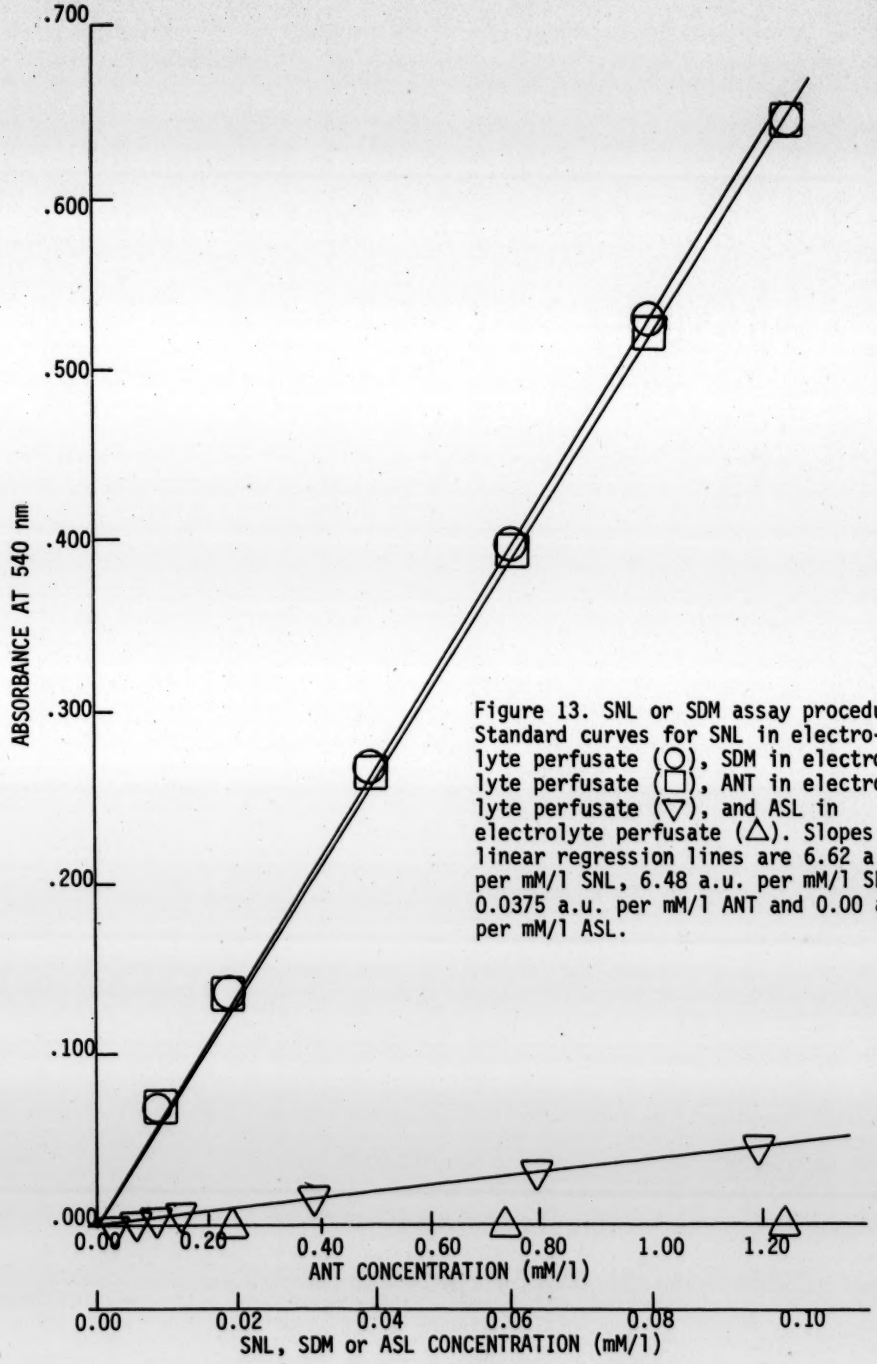


Figure 13. SNL or SDM assay procedure. Standard curves for SNL in electrolyte perfusate (○), SDM in electrolyte perfusate (□), ANT in electrolyte perfusate (▽), and ASL in electrolyte perfusate (△). Slopes of linear regression lines are 6.62 a.u. per mM/l SNL, 6.48 a.u. per mM/l SDM. 0.0375 a.u. per mM/l ANT and 0.00 a.u. per mM/l ASL.

N-4, acetylsulfanilamide (ASL)

Determination of ASL is performed by the identical procedure described for SNL or SDM after subjecting the sample to a treatment which frees the aromatic amino group by hydrolysis of the acetyl function.

A 1.0 ml aliquot of the perfusate sample is pipeted into an eight inch test tube and 1.0 ml 0.8 N HCl is added. The tube is stoppered and placed in an oil bath maintained at 100° for one hour. After the tubes are allowed to cool to room temperature, the procedure described for assay of SNL or SDM is followed beginning with the addition of 0.5 ml 0.8 N HCl to the addition of the color reagent. This step is followed by addition of 4.0 ml 0.1 N HCl and additional 4.0 ml increments as is necessary to adjust the absorbance to below a value of 0.600. The absorbance is read at 540 nm exactly two minutes after addition of the color reagent.

During the heating step, care must be taken so that water vapors do not escape the tube. With the use of eight inch glass stoppered tubes, the water vapors condense on the walls of the tube and are continuously refluxed.

Absorbances produced by solutions containing ASL are proportional to the sample concentration over the range of 0.01 to 0.08 mM/l, figure 14. Absorbances produced by SNL, SDM and ANT solutions are also proportional to sample concentrations and are not significantly different than the absorb nces produced by the same solutions in the assay procedure for SNL or SDM, figures 13 and 14. The regression coefficients calculated for the standard curves of ASL, SNL, or SDM prepared by this

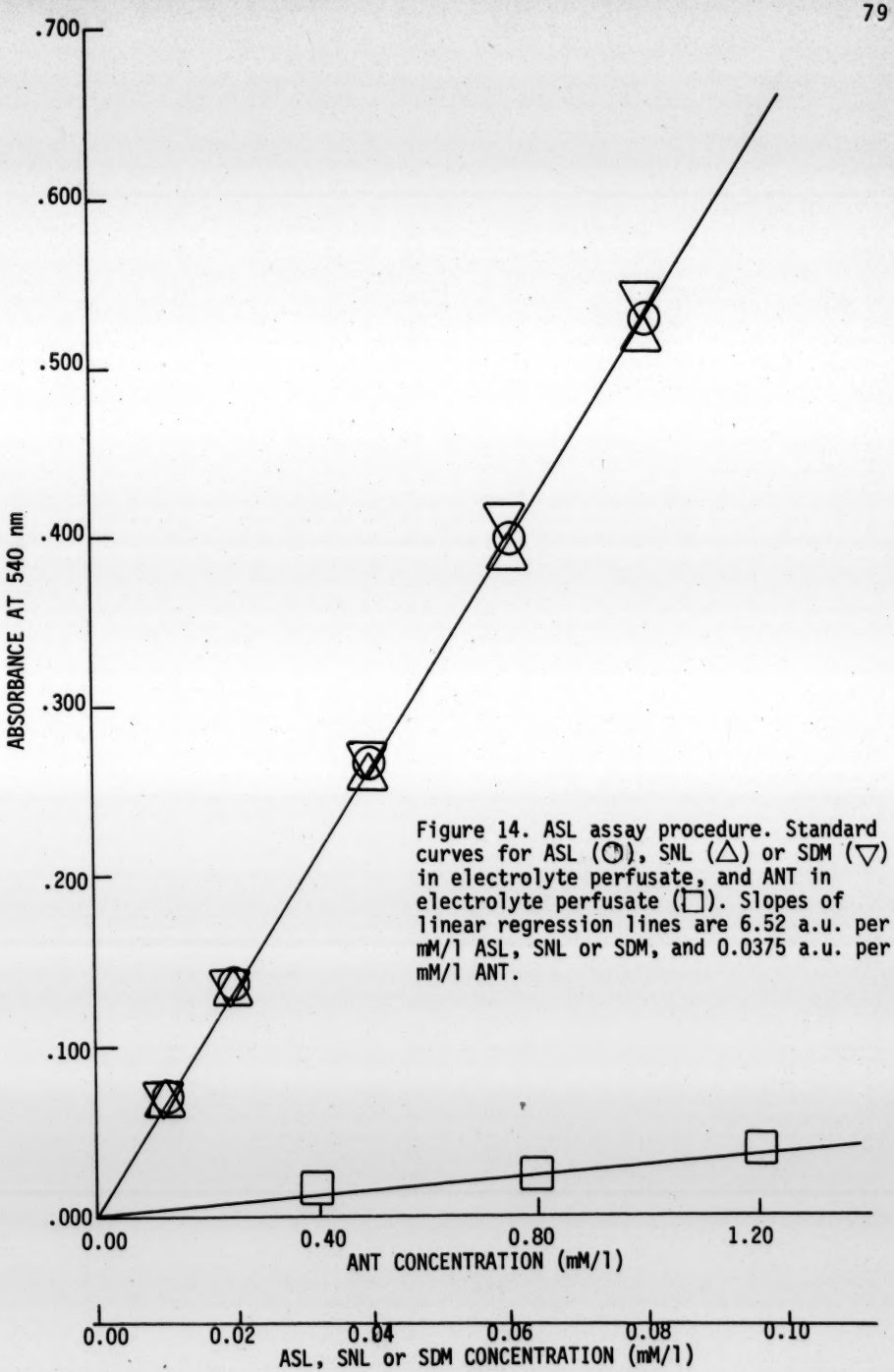


Figure 14. ASL assay procedure. Standard curves for ASL (○), SNL (△) or SDM (▽) in electrolyte perfusate, and ANT in electrolyte perfusate (□). Slopes of linear regression lines are 6.52 a.u. per mM/l ASL, SNL or SDM, and 0.0375 a.u. per mM/l ANT.

procedure are not significantly different from each other, nor from the regression coefficients for SNL or SDM standard curves prepared for SNL or SDM.

Determination of ASL concentration in samples containing ANT and SNL or SDM is made by subtracting the absorbance at 540 nm read after performing the procedure for SNL or SDM, from the absorbance read at 540 nm after performing the procedure for ASL on the same sample. The concentration of ASL in the sample is then calculated from the regression equation for the standard curve using the difference in absorbance readings.

There is no interference produced by the simultaneous presence of BSP, GLU, or DEX in the sample.

Measurement of Partition Coefficients

Partition coefficients between octanol and aqueous solutions at 37° are determined in the following manner.

Four 25.0 ml portions of an aqueous solution of each substance for which determination of partition coefficient is to be made, are warmed to 37° and pipeted into separate eight inch test tubes which can be fitted with ground glass stoppers. Into three tubes of each set, 25.0 ml octanol, which has been redistilled and warmed to 37°, is slowly pipeted down the wall of the tube such that the octanol does not mix with the aqueous layer. The fourth tube in each set serves as a control in the case there is degradation of the substance under study during the period of the determination.

Glass stoppers are tightly fit into each tube, the tubes are gently inverted, and the tops of the tubes are immersed in a molten mixture of beeswax and paraffin in order to insure a

watertight seal. The sealed tubes are clamped onto metal trays which are specially designed to fit onto a rocking device submerged in a water bath maintained at 37°. The rocking device is turned on and the speed is adjusted so that the rocking motion is gentle enough to minimize emulsification. After 36 hours, the tubes are removed from the tray and placed upright in the water bath where they remain an additional 24 hours to insure separation of the two phases. After this period, the stoppers are removed and the octanol layer removed by aspiration and discarded. An aliquot of each aqueous phase is pipeted into a test tube and refrigerated until ready for assay.

The concentration of the substance determined by assay of the aqueous solution to which no octanol is added but is otherwise treated as the portions of the solution to which control is added, is taken as the initial concentration of the substance in the aqueous layer.

Since the volumes of both the aqueous and octanol layers are equal, the concentration on the substance in the octanol layer is simply the difference in the initial and final concentrations of the substance in the aqueous layer.

The partition coefficient is found by calculating the ratio of the concentration of the substance in the octanol layer to the concentration in the aqueous layer.

FUNCTIONAL CAPABILITY OF THE PERFUSION APPARATUS AND PHYSIOLOGICAL PERFORMANCE OF PLACENTAS PERFUSED EX VIVO

Experiments

Development of Perfusion Methodology

The initial experiments performed placed emphasis on the perfection of the methodology described in the Materials and Methods section. This methodology includes: 1) the techniques for procurement, examination, and preparation of placentas; 2) the sequence of steps to be followed in mounting placentas in the apparatus and initiating perfusion; 3) methods for measurement of pressure, flows and reservoir volumes; 4) procedures for collecting samples.

In addition to the primary purpose of developing the methodology to be used in the subsequent experiments, experiments were performed using those placentas which had been successfully cannulated and perfused. These experiments were designed: 1) to test for leakage from the apparatus and for leakage between the placental and amniotic chambers of the apparatus; 2) to determine if perfusate flow rates and pressures within the placental vessels and compartments during ex vivo perfusion were within limits of the values reported to exist in vivo; and 3) to establish some preliminary data concerning the disposition of sulfobromophthalein (BSP), blue dextran (BD), and antipyrine (ANT), after their addition to either the maternal or fetal circuit of the perfused placenta. Table II lists each of the placentas perfused and the purpose of each experiment.

Table II

Developmental Experiments

Placenta #	Purpose of Experiment	Remarks
1	To test leakage from apparatus and between chambers	Fetal circuit not perfused BD* added to maternal circuit
17	To measure flow rates and pressures	
21	To measure flow rates and pressures	
24	To measure flow rates and pressures	
25	To measure flow rates and pressures	
29	To study disposition of BSP	Only fetal circuit perfused. BSP added to fetal circuit. Sampled FA, FV*
32	To measure flow rates and pressures	BSP added to fetal circuit, sampled FA, FV, MA, MV, AF
36	To measure flow rates and pressures, to study disposition of BSP and BD	BSP and BD added to fetal circuit, sampled FA, FV, MA, MV, AF
37	To measure flow rates and pressures, to study disposition of ANT and BD. To test use of rubber sheet	BD added to fetal circuit, ANT added to maternal circuit, sampled FA, FV, MA, AF
41	To measure flow rates and pressures, disposition of BSP, BD, and ANT	ANT added to fetal circuit, BSP and BD added to maternal circuit, sampled FA, FV, MA, MV

*abbreviations
BD = blue dextran
BSP = sulfobromophthalein
ANT = antipyrine
FA = fetal reservoir
FV = fetal venous circuit
MA = maternal reservoir
MV = maternal venous circuit
AF = amniotic fluid chamber

In this group of experiments, Ringer's solution was used as perfusate for both the maternal and fetal circuits as well as for amniotic chamber fluid. The perfusates were not oxygenated and no attempt was made to control pH of either perfusate or of the amniotic chamber fluid.

The fetal membranes of placentas 29 and 37 were torn from the placenta during delivery precluding their use to form a seal separating the placental and amniotic fluid chambers. For this reason, only the fetal circuit of placenta 29 was perfused to study the disposition of BSP when present in the fetal circuit. Both circuits of placenta 37 were perfused by use of the rubber sheet which is described in the Materials and Methods section. The rubber sheet was used as a substitute for the fetal membranes. The fetal membranes of the other placentas perfused were intact.

Amounts of BSP, BD, and/or ANT in maternal and fetal circuits at each sampling time point were calculated for placentas 29, 32, and 37. The amount calculated in each circuit included the amounts contained in the arterial and venous priming volumes for the placenta and the apparatus. The priming volumes used for these calculations were 150 ml for the maternal arterial circuit, 65 ml for the fetal arterial circuit, 100 ml for the maternal venous circuit, and 25 ml for the fetal venous circuit. The amounts in each circuit and the amount not accounted for in both circuits (called the extravascular amount or EX) were calculated as percentages of the total amount in the system. In order to correct for recoveries of each substance, and for amounts removed by sampling, the total amount in the

system was taken as the amount calculated from the assayed concentration of the solution prior to perfusion, and was corrected at each time point by subtracting the amounts removed from the system by previous sampling.

Amounts in maternal and fetal circuits were not calculated for placenta 41 because volume measurements were not reliable in that experiment.

Evaluation of Placental Viability

Once confidence had been acquired in the perfusion methodology, three placentas (42, 43, and 45) were perfused under conditions more closely resembling *in vivo* conditions. These experiments were performed in order to evaluate the viability of the *ex vivo* perfused placentas.

Improvement of physiologic conditions of the perfusion was brought about by: 1) the use of buffered electrolyte solutions for amniotic chamber fluid and perfusates, 2) the addition of dextrose (GLU) to both maternal and fetal perfusates, 3) oxygenation of maternal perfusate, and 4) continuous adjustment of maternal and fetal perfusate pH. The composition of the buffered electrolyte solutions is presented in the Materials and Methods section.

The metabolic activity of the perfused placentas was studied by monitoring glucose disappearance, and by monitoring the appearances of lactate and pyruvate in the perfusates.

Low molecular weight dextran (DEX) was added to the fetal circuit in each of these experiments. The molecular weight of DEX (average = 40,000) is low enough to provide osmotic efficiency, and high enough so as not to be expected to

escape the fetal circuit via the capillaries. The purpose of providing a fetal to maternal osmotic pressure gradient with DEX was to produce opposition to fluid transfer in the fetal to maternal direction.

Flows, pressures, reservoir volumes, and perfusate pHs were measured periodically during the experiments. Samples were collected at intervals from the fetal and maternal reservoirs during the perfusion of all three experiments. Additional samples were collected from the fetal and maternal venous circuits during perfusion of placentas #43 and #45. Samples were also collected from the fluid contained in the amniotic fluid chamber at the conclusion of each of these two experiments.

Each sample was deproteinated immediately upon collection. The supernatants were refrigerated until subsequent analysis for GLU, DEX, lactate, and pyruvate.

Results and Discussion

Availability of Suitable Placentas

Considerable difficulty was encountered in the collection of placentas suitable for use in these experiments. Table III lists the problems and successes of the first forty-one placentas obtained from the delivery room.

No records were kept of the number of placentas not obtained from the delivery room due to: 1) lack of notification by the delivery room staff, 2) the occurrence of deliveries at inopportune times, or 3) the possible presence of a pathological condition in the mother, fetus, or placenta.

TABLE III

Suitability of Placentas for Perfusion

Placentas Rejected on Visual Inspection		14
Lacerations on decidua	11	
Punctured or lacerated umbilical cord	3	
Diameter of placenta too large for apparatus	1	
Placentas Not Rejected on Visual Inspection		27
Unsuccessful cannulation of umbilical vessels	7	
Successful cannulation unsatisfactory perfusion	11	
Cannula slippage	5	
Gross fetal perfusate leakage	6	
Successful cannulation acceptable perfusion	9	
Total		41

Slippage of cannulas from the umbilical vessels before and during perfusion was a major problem, accounting for eighteen of the thirty-two failures. The smooth walled polyethylene tubing which was used for cannulas in these experiments could not be adequately anchored within the vessels to prevent their sliding out under the pressure of perfusion. Glass cannulas, made with a constriction in the wall around which a tie could be made were also tried. They, too, tended to slip out of the vessels under pressure. Finally, Sim's tips were used to cannulate the umbilical vessels. No slippage occurred when two or three ties with braided umbilical tape were made around the umbilical cord whose vessels were cannulated with Sim's tips.

Those placentas with gross fetal leakage consisted of placentas which had, upon initiation of perfusion, very little or no fetal perfusate return. Further inspection of these placentas showed decidual lacerations or punctured umbilical cords which were not detected on initial visual inspection.

Aside from the cannulation problem, which was corrected as discussed above, problems similar to those listed in table III were encountered throughout this work and severely limited the scope of research which could be successfully completed.

Other workers attempting to perfuse the human placenta have reported similar problems and "batting averages" in the collection of suitable placentas. Nesbitt and co-workers reported that only 10% of the placentas which reached their laboratory were satisfactory for perfusion of maternal and fetal circuits (138). Shier and co-workers stated that of 26 placentas which appeared to be suitable for perfusion and were attached to their perfusion apparatus, only twelve were considered to be successful (140).

Tests for Leakage from the Apparatus and for Leakage Between Chambers of the Apparatus

The maternal circuit of placenta 1 was perfused for 75 minutes in the apparatus whose amniotic fluid chamber was filled with Ringer's solution. The cannula connectors of the apparatus, which were unused since the fetal circuit was not perfused, were stoppered to prevent leakage of fluid from the amniotic fluid chamber.

During the experiment, there was no change in the level of fluid contained in the amniotic fluid chamber, nor was there

visible evidence of fluid leakage from any part of the perfusion apparatus or from the manifold. Analysis of the solution drained from the amniotic fluid chamber after ending the perfusion showed no trace of BD.

These observations, and those of subsequent experiments, during which no leakage was observed, provided confirmation that the apparatus was watertight and that the fetal membranes, or rubber sheet which replaced the fetal membranes in their absence, were capable of providing a leakproof seal between the placental and amniotic chambers.

Flow Rates and Pressures

Values representing the range of flow rates and pressures measured during perfusion of nine placentas are shown in table IV. During the perfusions, the intervillous space pressure was always equal to the amniotic fluid pressure.

Maternal arterial pressures tended to remain nearly constant during any one experiment, within a range of 9 mm Hg.

Fetal arterial pressures remained nearly constant throughout an experiment unless there was an increase in the rate of fetal to maternal transfer of fluid. At such times, a rise in fetal arterial systolic and diastolic pressures occurred concurrent with the increase in the rate of fetal to maternal fluid flow. At least a small amount of fluid was constantly being transferred from the fetal to maternal circuit in all experiments.

Differences in the pressures measured in each of the two umbilical arteries of a particular placenta varied among individual placentas from no difference at all in systolic and

diastolic pressures, to differences of 10 mm Hg in diastolic pressures and 20 mm Hg in systolic pressures. Both maternal and fetal venous pressures were adjusted to maintain constant values throughout each experiment.

In all experiments, the maternal pump was adjusted to deliver 600 ml/min with a stroke rate of 60/min. The fetal pump was set to produce 60 pulsations/min and delivered 114 ml of perfusate per minute with the tubing employed.

TABLE IV

Range of Flow Rates and Pressures Measured in Nine Placentas

Measurement		Maternal	Fetal
Flow rate,	ml/min	600	114
Arterial systolic pressure,	mm Hg	55-83	59-110
Arterial diastolic pressure,	mm Hg	42-70	24-70
Venous pressure,	mm Hg	7.4-19.9	14.7-29.4
Intervillous space pressure,	mm Hg	23.5-29.4	---
		Amniotic Fluid	
Amniotic fluid pressure,	mm Hg	23.5-29.4	

The maternal flow rate, and the ranges of fetal and maternal arterial and venous perfusion pressures measured in these studies are in the range of those reported by Krantz, et al., during ex vivo perfusion of the human placenta (123), and fall within the range of the values estimated to occur in vivo (37,59,123,162-165).

The value of 114 ml/min for the fetal perfusate flow rate is within the range used by Krantz, et al. (123), but it is far below the value of 495 ml/min estimated by Dawes (59) to be the umbilical blood flow rate occurring in term human fetuses. Attempts to increase fetal perfusion flow rate resulted in high fetal arterial pressures and rapid fetal to maternal fluid transfer. Similar response to high fetal perfusion rates have been experienced by other investigators (138).

Equilibration of intervillous pressure with amniotic fluid pressure occurs in this preparation as well as in vivo (165). Amniotic fluid pressures higher than the 10-20 mm Hg reported to exist in vivo when the uterus is resting (165) are required in this preparation to keep the placenta resting on the perfusion tubes at the base of the placental chamber (123). The 23-30 mm Hg amniotic fluid pressures used in these studies are, however, much less than the value of 70 mm Hg reportedly reached in vivo when the uterus is contracting (165). An apparatus recently designed by Nesbitt, et al., eliminates the necessity for high amniotic fluid pressures and allows the placenta to be perfused with a minimum of 15 mm Hg pressure in the amniotic fluid chamber (138); however, this research was completed before publication of the Nesbitt, et al., report.

Physiological Performance of Placentas 29, 32, 36, 37 and 41 and Preliminary Studies of the Disposition of Sulfobromophthalein, Blue Dextran, and Antipyrine

Results.--Reservoir volume measurements and concentrations of sulfobromophthalein (BSP), blue dextran (BD), and antipyrine (ANT) determined in samples collected during

perfusions of placentas 29, 32, 36, 37, and 41 are plotted in figures 15-19 at the times at which the measurements were taken or the samples collected.

Pressure measurements read during the experiments are listed in table V along with average rates of fetal fluid loss. The average rate of fetal fluid loss at any time point was calculated by dividing the difference in fetal reservoir volume measurements of the previous and subsequent time points, by the interval of time between the previous and subsequent time points.

Amounts of BSP, BD, and ANT accounted for in the maternal or fetal circuits and in the amniotic chamber fluid, and the amounts of each substance not accounted for in the circuits, are shown in figures 20-23 as a percentage of the total amount in the system.

Fetal to maternal fluid flow and perfusion pressures.--It can be seen from the data presented in figures 15-19 and table V, fetal to maternal fluid transfer at rates of 1 to 30 ml/min occurred during the course of each experiment. Most of the fluid lost from the fetal circuit was accounted for by simultaneous increases in maternal reservoir fluid volume. For example, the total volumes of fluid unaccounted for after perfusion of placentas 36 and 37 were 40 and 55 ml, respectively. These volumes represented 2.30 and 3.13% of the total perfusate volumes. Although these volumes were within the limit of error of the volume measurements, they may also represent edema fluid taken up by the placenta.

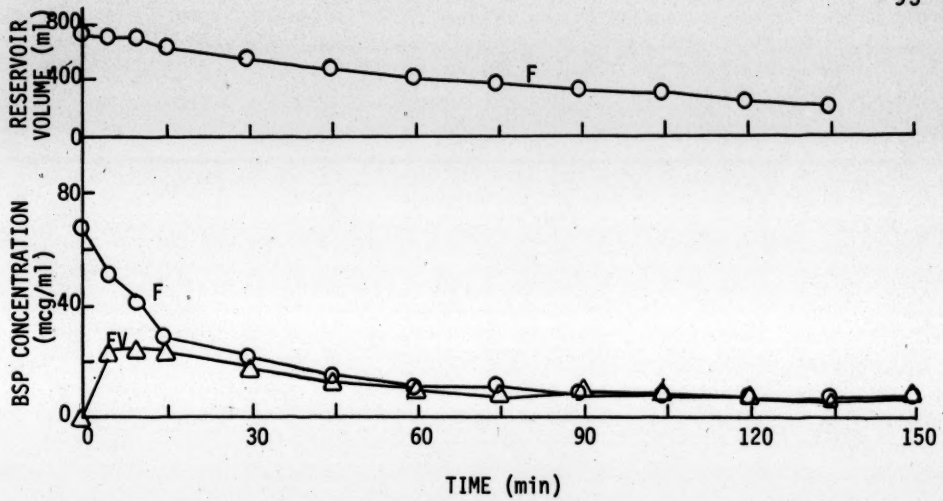


Figure 15. Fetal reservoir volume and concentrations of BSP in samples from the fetal reservoir (F) and from the fetal venous circuit (FV) of placenta 29.

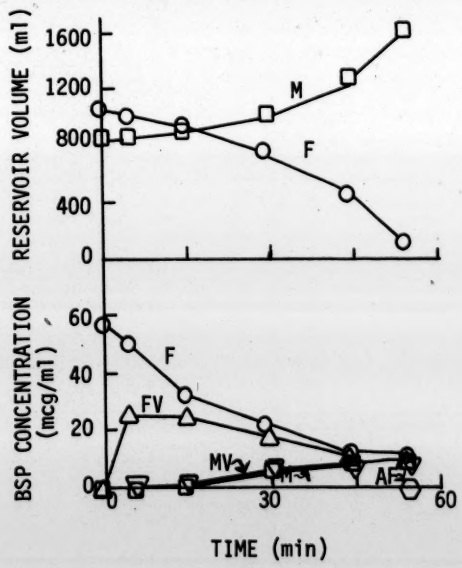


Figure 16. Reservoir volumes and concentrations of BSP in samples from fetal (F) and maternal (M) reservoirs, fetal venous circuit (FV), maternal venous circuit (MV), and amniotic chamber fluid (AF) during perfusion of placenta 32.

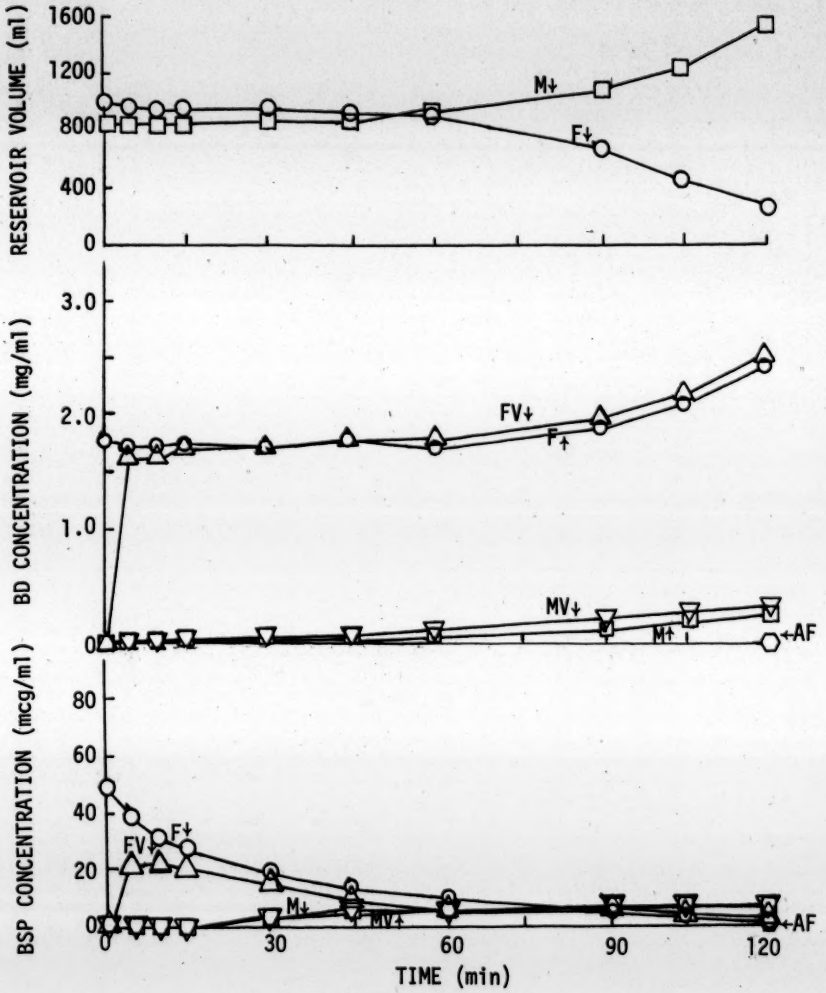


Figure 17. Fetal and maternal reservoir volumes, and concentrations of BSP and BD determined in samples from the fetal reservoir (F), maternal reservoir (M), fetal venous circuit (FV), maternal venous circuit (MV), and amniotic fluid chamber (AF) during perfusion of placenta 36.

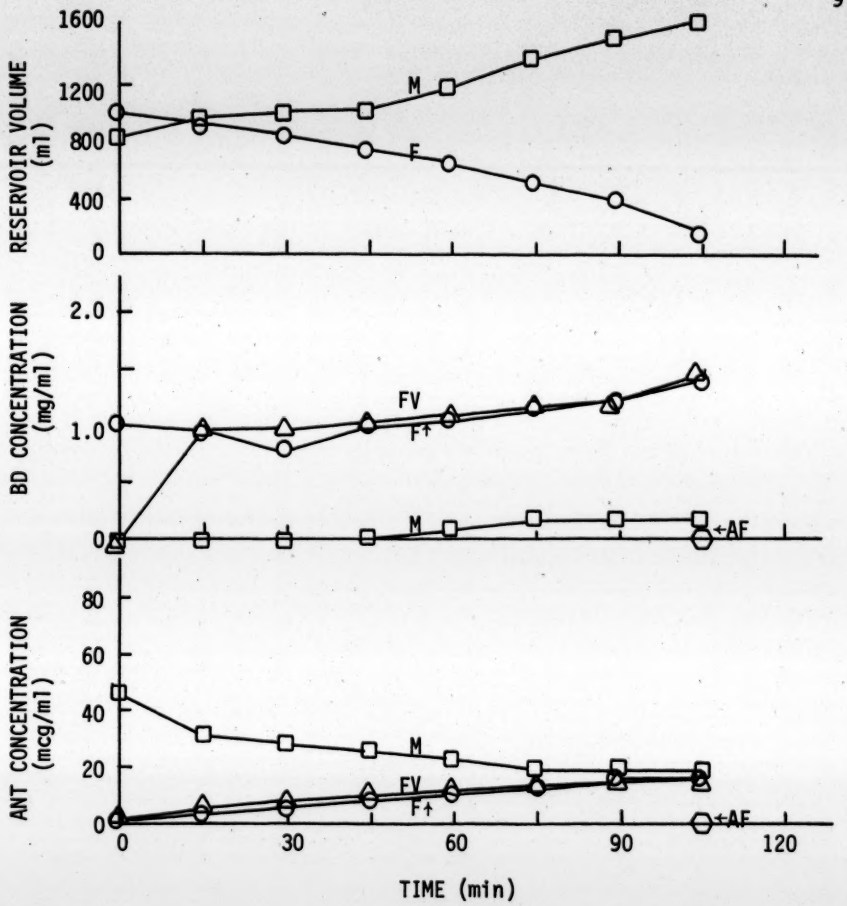


Figure 18. Fetal and maternal reservoir volumes and concentrations of BD and ANT determined in samples from the fetal reservoir (F), maternal reservoir (M), fetal venous circuit (FV), and amniotic chamber fluid(AF), during perfusion of placenta 37.

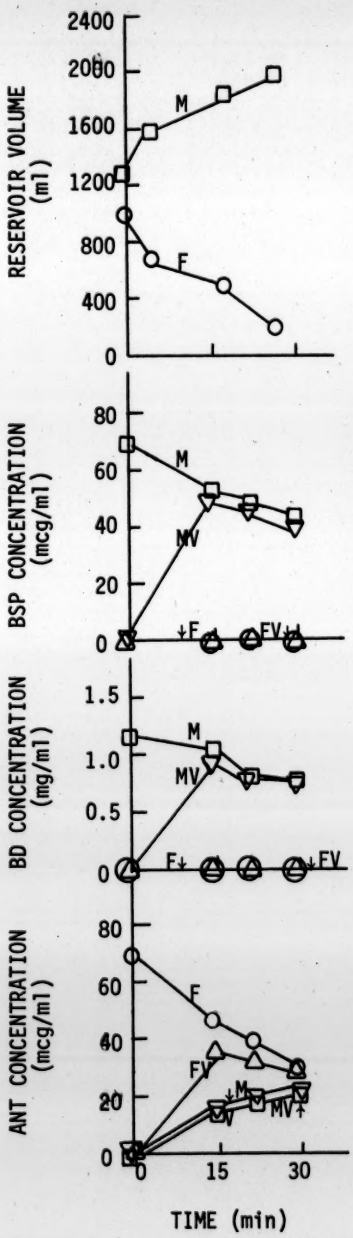


Figure 19. Fetal and maternal reservoir volumes and concentrations of ANT, BSP and BD in samples from the fetal reservoir (F), maternal reservoir (M), fetal venous circuit (FV), and maternal venous circuit (MV) during perfusion of placenta 41.

TABLE V

Average Rates of Fetal Fluid Loss and Pressures Recorded during Perfusion of Placentas 32, 36, 37, and 41

Placenta #	Experimental time	Rate of fetal fluid loss	Pressures (mm Hg) Fetal Arterial 1	Fetal Arterial 2	Fetal Venous	Maternal Arterial	Amniotic fluid
	min	ml/min					
32	-15	---	90/59	59/48	---	62/45	27.4
	8	6.0	79/24	60/35	---	62/44	24.5
	20	10.7	86/25	66/50	---	65/50	25.4
	40	20.0	85/25	65/55	---	65/50	27.2
	50	36.7	85/25	75/65	---	60/45	28.7
36	- 5	---	72/29	70/38	14.7	57/47	27.0
	2	1.0	70/30	70/38	14.7	57/47	26.8
	20	1.0	73/28	73/36	14.7	55/48	27.0
	35	1.7	77/29	75/50	14.7	55/47	27.2
	50	3.7	80/34	80/39	14.7	56/49	27.2
	65	5.8	85/36	80/40	14.7	56/49	27.2
	80	7.6	95/39	90/45	14.7	59/50	28.7
	95	13.0	95/36	95/36	14.7	53/50	23.5
	110	14.0	98/42	98/52	14.7	55/48	24.6
	125	---	105/45	103/53	14.7	50/48	23.9
37	- 5	---	90/38	73/43	18.3	78/60	28.3
	5	5.0	88/26	70/35	29.4	---	25.4
	30	6.0	90/29	78/42	14.7	78/58	24.6
	45	6.7	92/30	80/42	11.0	75/54	25.0
	60	8.0	92/31	80/35	11.0	83/60	25.7
	75	9.0	94/34	83/42	18.3	80/70	25.7
	90	12.3	102/34	90/42	11.0	80/63	25.7
	105	---	108/47	97/56	11.0	80/64	26.8
41	-20	---	90/36	68/38	22.1	62/50	25.0
	18	22.7	90/38	75/48	22.1	63/53	25.7
	27	---	90/36	70/45	22.1	64/50	25.7

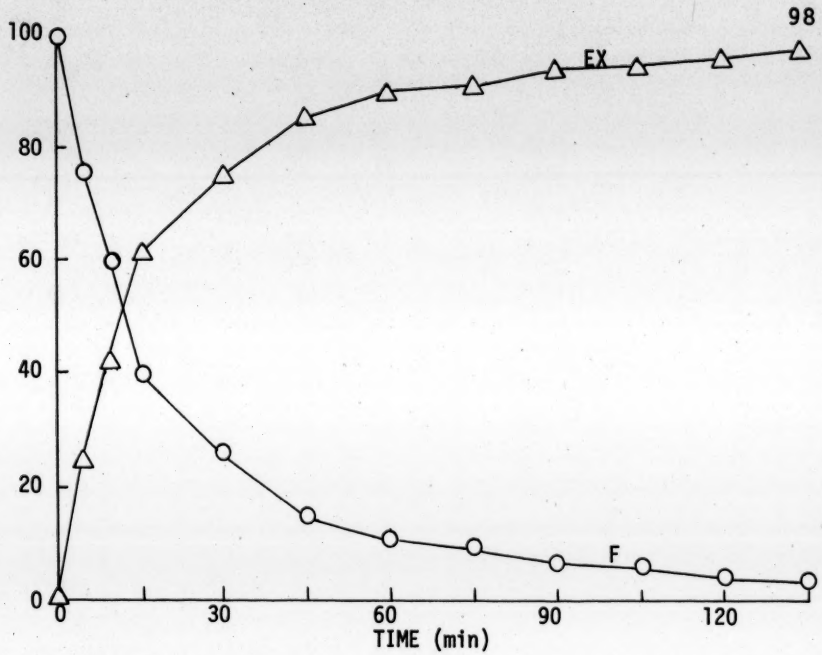


Figure 20. Percentage of total BSP accounted for in the fetal circuit (F), and unaccounted for (EX) during perfusion of placenta 29.

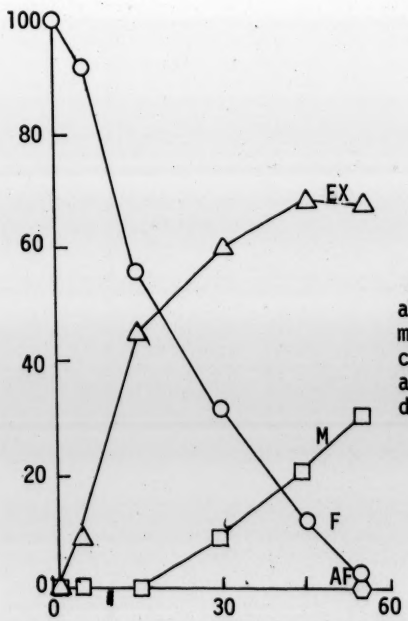


Figure 21. Percentage of total BSP accounted for in fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF), and BSP not accounted for in perfusates (EX) during perfusion of placenta 32.

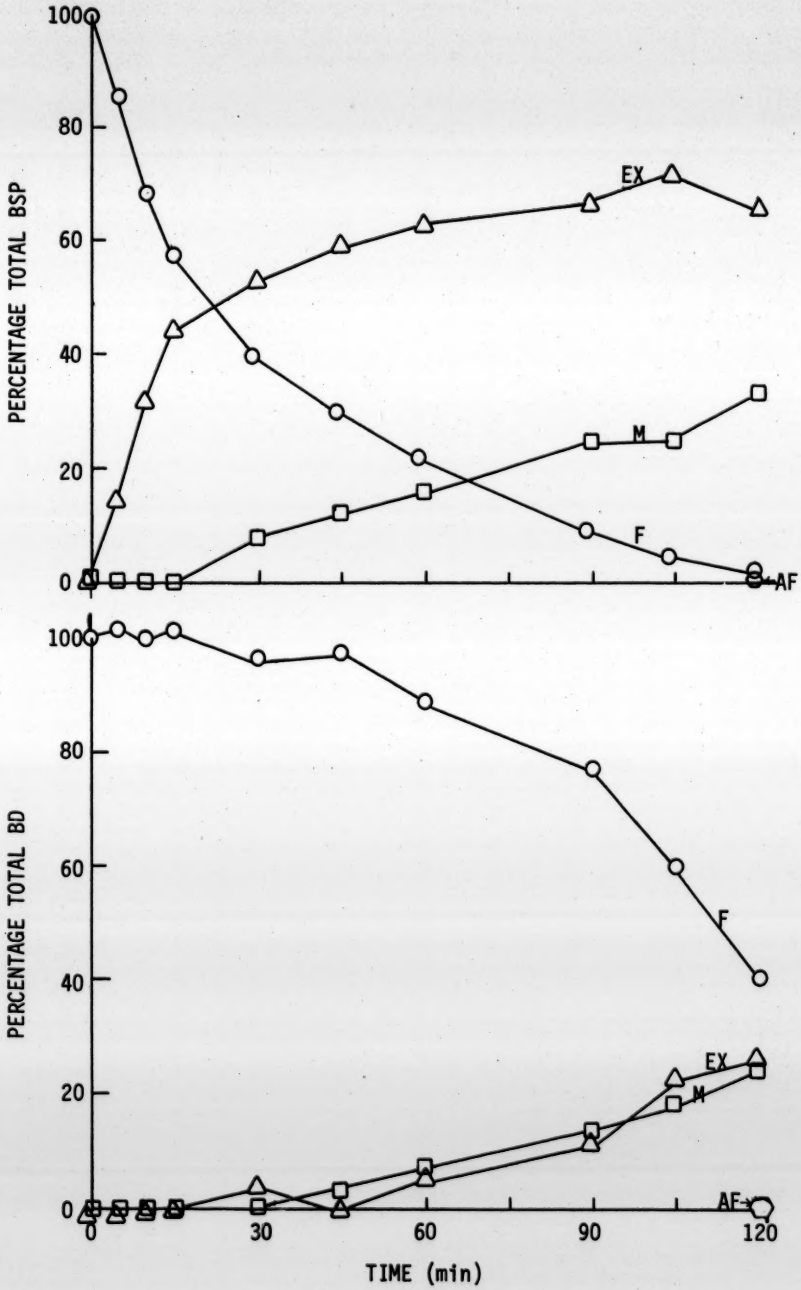


Figure 22. Percentages of total BSP and BD accounted for in the fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF), and amounts not accounted for in perfusates (EX) during perfusion of placenta 36.

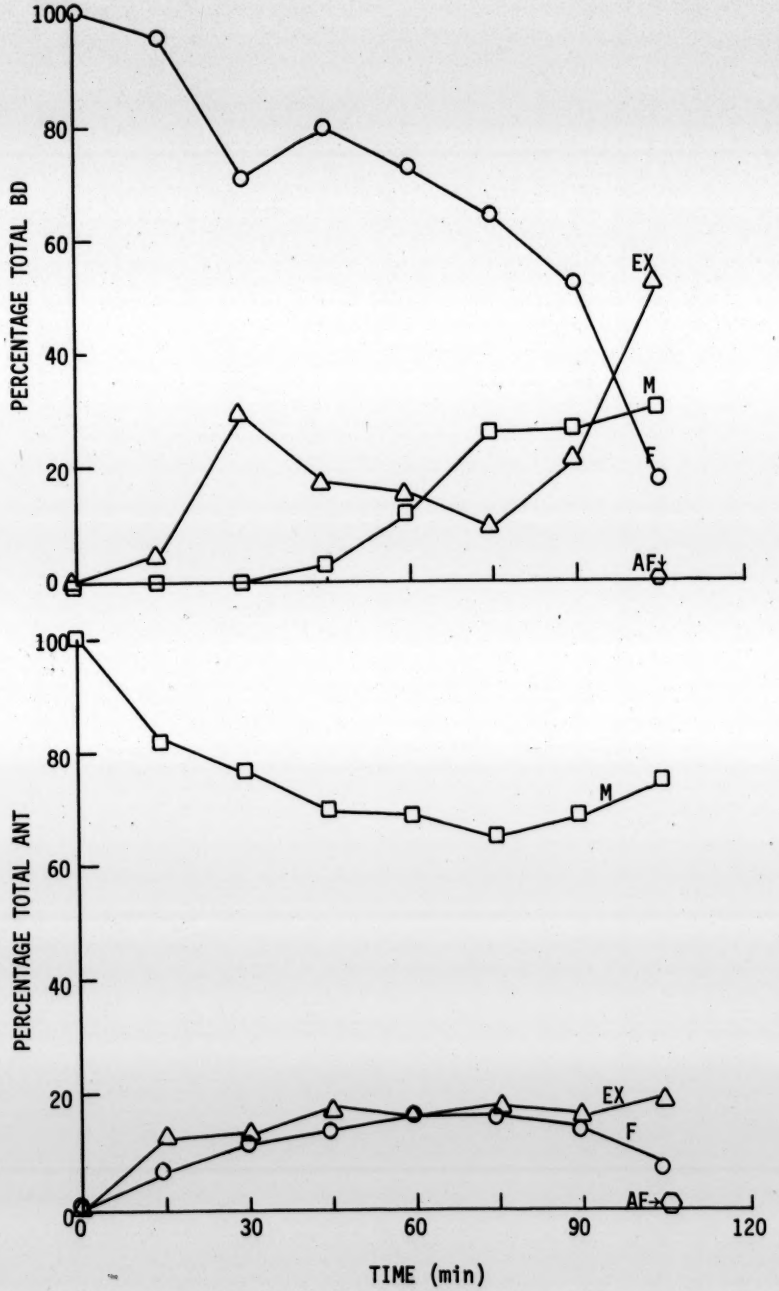


Figure 23. Percentages of BD and ANT accounted for in the fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF), and amounts not accounted for in perfusates (EX) during perfusion of placenta 37.

The data listed in table V show that some fetal to maternal fluid flow occurs even when pressures measured in the fetal and maternal circuits and in the amniotic fluid chamber are within ranges estimated to occur in vivo. It is apparent from the data of each experiment that increases in the rate of fetal to maternal fluid flow occur simultaneously with increases in fetal arterial systolic and diastolic pressures in at least one of the fetal arteries. These increases in fetal arterial pressures occur while fetal venous, maternal arterial, and amniotic fluid pressures are constant.

Some fetal to maternal flow of fluid is to be expected as a result of the necessity of the pressure within the fetal capillaries to exceed intervillous space pressures in order to prevent collapse of the fetal vessels. The probability of net fetal to maternal flow of water across the placenta in vivo in response to in vivo hydrostatic and osmotic pressure gradients has been discussed in the Introduction.

Fetal to maternal transfer of fluid is a problem commonly encountered by other investigators perfusing the human placenta ex vivo.

Panigel, upon perfusing only the fetal vessels of placentas, reported a constant water leakage from fetal vessels which is negligible during the first few hours, but which becomes more marked as the perfusions continue (121). Krantz and co-workers state that fluid is transferred from fetal to maternal circuits in almost all placentas perfused at rates up to and exceeding 2.5 ml/min (123). Nesbitt and co-workers found transfer of fluid a continuing problem in perfusing maternal and

fetal circuits of the placenta in their apparatus (138). They state that with suitable preparations, the transfer rate of fluid from fetal to maternal circuit is less than 2 ml/min. During some perfusions there would be little or no fluid transfer for several hours. After about four hours of perfusion they note a dramatic increase in the rate of fluid transfer, within a 30 minute period, to a rate greater than 10 ml/min. Data presented by Hamrin and co-workers from their perfusion studies indicated fetal fluid volume loss at rates approaching 16 ml/min (141). Finally, Keller indicated leakage to be a constant problem during ex vivo placental perfusion, but presented no data to indicate the extent of the problem (166).

With the exception of the first 50 minutes of perfusing placenta 36, fetal to maternal fluid flow rate exceeded the 2 ml/min reported by Nesbitt, et al., and the 2.5 ml/min reported by Krantz, et al. There are two possible reasons for such excessive rates of fetal flow.

One possible reason for excessive fetal to maternal fluid flow is bulk flow of fluid from damaged fetal capillaries caused by mechanical damage to the placenta produced by the trauma of labor and delivery. If excessive, such leakage would be evident on initiation of the perfusion accompanied by low fetal arterial pressure. On the contrary, the rapid flow of fluid which occurred in these experiments were accompanied by increased pressures. This does not rule out the possibility of some of the fluid transfer caused by bulk flow of fluid from damaged capillaries, but, it is unlikely that this is a major cause. In addition, those placentas exhibiting visible damage,

and those demonstrating gross leakage on initiation of perfusion were discarded.

A second possible reason for fetal to maternal fluid flow is an increase in permeability due to the lack of globulins in the perfusate. Panigel points out, in his review of placental perfusion experiments (121), that water leakage may be reduced, but not prevented by the use of Dextran in an electrolyte perfusate in lieu of plasma. The globulins present in plasma presumably maintain the integrity of the endothelial membrane. The lack of globulins in those experiments may have contributed to fetal to maternal fluid flow.

A third, likely reason for such excessive fetal to maternal fluid flow during these experiments, is the consequence of hypoxia. Hypoxia is known to produce cellular swelling which precedes cellular destruction (167). Swelling of cells surrounding the capillaries increases resistance in the vessels, thus causing perfusion pressures to rise. Such increases in fetal arterial pressure without concurrent increase in intervillous pressure raise the fetal to maternal hydrostatic pressure gradient, which, in turn, increases the filtration rate of fluid, as discussed in the Introduction.

There is no doubt that extensive hypoxia and ischemia occurred during these experiments in which no oxygen or nutrient was added to the perfusates.

Disposition of sulfobromophthalein (BSP).--It was, at first, thought that BSP could be employed as a marker to detect bulk flow of fluid from fetal to maternal circuits. Other investigators perfusing the human placenta ex vivo have used BSP

for this purpose (135,138). Moreover, it has been reported that after injection of BSP in women during labor, only insignificant concentrations of the drug were detected in fetal blood and in placental tissues (71-73).

However, in this work, as seen in figures 20-22, BSP rapidly disappeared from the fetal circuit during experiments 29, 32, and 36 in which BSP was contained in the fetal perfusate at the start of perfusion.

It is apparent, from the rapid drops of BSP concentration in the fetal circuit during these experiments (figures 15-17), that BSP left the fetal circuit more rapidly than did water; thus, disappearance of BSP from the fetal circuit was due to some mechanism in addition to solvent drag. During the first 50 minutes of perfusion of placenta 36, in which fetal fluid loss was relatively small, disappearance of BSP was still quite rapid (figures 17 and 22).

Further examination of the data shown in figures 21 and 22 shows that the rate of appearance of BSP in the maternal circuit was quite slow, and, that no more than one-third of the BSP which left the fetal circuit, could be accounted for in the maternal circuits of placentas 32 and 36. BSP was not detected in samples from the fluids contained in the amniotic fluid chamber at the conclusion of these experiments.

No conclusions could be made, at this point, regarding the disposition of BSP during perfusion of placenta #41 in which the compound was initially added to the maternal circuit. BSP was not detected in fetal perfusate samples collected during the 30 minutes of this experiment (figure 19). Calculations show

that the drop in maternal perfusate concentrations of BSP during the 30 minute period of perfusion, were not significantly different than the concentration drop expected by dilution of the maternal perfusate by the rapid influx of fetal fluid.

The possibilities of BSP removal from the fetal circuit by the plastic components of the fetal reservoir, by the plastic tubing used in the fetal circuit, or as a result of chemical instability of BSP during the perfusion have not been ruled out at this point to account for its rapid disappearance from the fetal circuit. It is unlikely that BSP is being removed by biotransformation occurring in the placenta since biotransformation has been shown not to occur when BSP is incubated with placental homogenates in vitro (168).

It is worthy to note that, at plasma concentrations below 300 mcg/ml, BSP is bound to plasma albumin to an extent greater than 99.9% (169). Szabo, et al. (133), who used BSP as a marker for leakage of fluid from the fetal circuit did not detect the compound in the maternal circuit in the absence of bulk fluid flow. Nesbitt and co-workers (138) similarly found BSP to be satisfactory for use as a marker for leakage. However, in both of these studies, diluted human blood was used as perfusion media, and, in one case (135), bovine serum albumin was also added to the perfusate.

Thus, it is not unlikely that BSP would rapidly escape the fetal circuit via the capillaries of the fetal circuit, during these experiments using a protein-free perfusate. The molecular weight of BSP is 838 and molecules as large as inulin, whose molecular weight is 5000, are known to pass through

capillary pores without hindrance (39). On the other hand, plasma albumin, to which BSP is strongly bound, escapes intact capillaries only with great difficulty (39).

It is also not unlikely that a large proportion of the BSP which escapes the fetal circuit may accumulate within the placenta. Many body organs are known to bind BSP, especially the liver. Several proteins which have been isolated from these body organs have been shown to be responsible for the binding of BSP (170).

These possibilities of BSP disposition await further discussion after additional work is reported in a later section.

Although BSP was added to the fetal circuit of placenta 36 and net transfer took place in the fetal to maternal direction, it is of interest to note that an increasing maternal to fetal concentration gradient of BSP was established after the first hour of perfusion (figure 17). This phenomenon was a consequence of simultaneous filtration and diffusion as will be discussed in a later section of this study.

Disposition of blue dextran (BD).--BD was added to the fetal perfusates of placentas 36 and 37 at the start of each experiment. The data, figures 17 and 18, shows that little change in fetal perfusate BD concentrations occurred during the first minutes of perfusing placenta 36. After the first 60 minutes of perfusion of placenta 36 and throughout the perfusion of placenta 37, fetal perfusate concentrations of BD increased with time. Appearance of BD in maternal perfusates occurred after 30 minutes in each experiment. BD was not detected in amniotic chamber fluid samples collected after either experiment.

The data presented in figure 22 shows that during the first fifteen minutes of the perfusion of placenta 36, all of the BD was accounted for in the fetal circuit, while, during the same period, more than 40% of the BSP had left the fetal circuit. After the first fifteen minutes of perfusion of placenta 36 and throughout the perfusion of placenta 37, figure 23, BD left the fetal circuit. About 50% of the BD which left the fetal circuit can be accounted for in the maternal circuit in both experiments.

BD is a large molecular weight polymer of glucose, having an average molecular weight of 2,000,000 and a covalently linked chromophore. Since molecules of much smaller weight, such as human serum albumin (MW 69,000) penetrate capillary walls only with great difficulty (39), retention of BD in the intact fetal circuit is to be expected.

One must be concerned about the stability of the chromophore on the molecule since the assay method measures only the chromophore and not the dextran molecule. Separation of the chromophore has been shown to take place only upon prolonged incubation with dextranase-secreting bacteria (171-172). BD has been successfully employed to estimate fluid volumes of fish intestines and dog stomachs and has been shown not to be absorbed through membranes lining those sites (171,173).

It may, therefore, be safely assumed that BD will leave the fetal circuit and appear in the maternal circuit only upon disruption of the fetal capillaries and the membranes separating the fetal capillaries from the maternal intervillous space.

With this assumption, it can be concluded that, during the first 15 minutes of perfusing placenta 36, the 1 ml/min

fetal to maternal fluid flow is not the result of mechanical or hypoxic damage to the organ; rather, it is the result of a "normal" fetal to maternal hydrostatic pressure gradient. At times after the first 15 minutes perfusion of placenta #36, BD begins to leave the fetal circuit and appear on the maternal side, figures 17 and 22, concurrent with the increases in fetal to maternal fluid flow rates, and pressures seen in table V. Thus, at least part of the increased fetal to maternal fluid flow rate may be attributed to the development of hypoxic damage to the tissues or mechanical damage caused by the increased fetal arterial pressures, resulting in direct leakage of fluid from fetal to maternal circuits. This leakage of BD is seen throughout perfusion of placenta 37 (figures 18 and 23), for which fetal to maternal fluid flow rates and fetal arterial pressures were high throughout the experiment.

The observation that BD concentrations in the fetal circuit increase during periods of high fetal to maternal fluid flow, indicate that water is transferred more rapidly than BD; Thus, fluid flow is caused by both filtration of fluid through intact capillaries, and by bulk leakage of perfusate.

Thus, it appears that BD is a suitable indicator to monitor for leakage of perfusate from fetal to maternal circuit of the perfused placenta.

No conclusion could be made regarding the maternal to fetal transport of BD. As with BSP, BD was not detected in fetal perfusate samples collected during the first 30 minutes of perfusion of placenta 41. Maternal perfusate concentrations of BD were not significantly different from concentrations expected

by dilution of the BD initially added to maternal perfusate, by influxing fetal perfusate.

Disposition of antipyrine (ANT).--ANT was initially added to the fetal circuit of placenta 41 and to the maternal circuit of placenta 37.

A rapid drop in fetal perfusate ANT concentration resulted when ANT was initially present only in the fetal circuit as shown in figure 19. This drop in concentration took place along with fetal to maternal fluid flow rates exceeding 15 ml/min. thereby demonstrating a rate of ANT transfer much faster than the rate of fluid transport.

Maternal to fetal transfer of ANT was not hindered by simultaneous fetal to maternal fluid flow rates greater than 5 ml/min as shown in figure 18. Fetal perfusate concentrations nearly equaled maternal perfusate concentrations after 105 minutes.

Figure 23 shows that about 50% of the ANT which left the maternal circuit could not be accounted for in maternal or fetal perfusates. ANT was not detected in amniotic fluid samples collected at the conclusion of experiment with placenta 37.

These preliminary experiments with ANT show that the transfer across the perfused placenta, in either direction, of a rapidly diffusible substance such as ANT, can be detected in spite of rapid fetal to maternal fluid flow.

Physiological Performance and Metabolic Activity and Viability of Placentas 42, 43, and 45

Table VI lists approximate rates of fetal fluid loss, pressures and perfusate pH measurements recorded during each

experiment. Concentrations of DEX, GLU, lactate and pyruvate determined from samples collected during each experiment are shown in figures 24-26. Reservoir volumes measured during perfusions and amounts of DEX calculated in the maternal circuit, the fetal circuit, and the amount not accounted for, expressed as percentages of the total amount are shown in figures 27 and 28 for placentas 43 and 45. Total amounts of GLU, lactate and pyruvate in maternal and fetal circuits are shown in figures 29 and 30 for placentas 43 and 45, respectively. Reservoir volumes could not reliably be measured for placenta 42; thus, amounts of each substance were not calculated.

Fetal to maternal fluid flow and perfusion pressures.-- High fetal to maternal fluid flow rates associated with high fetal arterial and venous pressures occurred throughout perfusion of placenta 42 (table VI).

Perfusion of placenta 43 began with fetal fluid loss greater than 6 ml/min (figure 27 and table VI). After the first 30 minutes of perfusion, fetal arterial pressures increased with an increasing rate of fetal fluid loss.

No measurable loss of fluid from the fetal circuit of placenta 45 occurred during the first hour of perfusion (figure 28 and table VI). During the second hour, fetal fluid was lost at increasingly rapid rates paralleled by rising fetal arterial pressures.

The significance of these findings with respect to mechanical and/or hypoxic damage which may have been suffered by these placentas will be discussed after consideration of further results.

TABLE VI

Average Rates of Fetal Fluid Loss, Pressures and Perfusate pH Measurements Recorded During Perfusion of Placentas 42, 43 and 45.

PLACENTA	EXPERIMENTAL TIME	RATE OF FETAL FLUID LOSS	PRESSURES						PERFUSATE pH	
			FETAL ARTERIAL		FETAL VENOUS	MATERNAL		AMNIOTIC FLUID	MATERNAL	FETAL
			1	2		ARTERIAL	VENOUS			
	min	ml/min	mmHg	mmHg	mmHg	mmHg	mmHg	mmHg		
42	0	--	120/34	--	--	--	--	26.1	7.4	7.4
	17	--	--	--	--	--	--	--	6.0*	7.5
	37	--	--	--	--	--	--	--	5.6*	7.3
	55	6.7	135/58	--	36.8	--	--	14.7	--	--
	80	--	--	--	--	--	--	--	6.9*	7.4
43	0	6.3	--	--	--	--	--	--	7.4	7.3
	4	--	--	--	--	--	--	--	7.3	7.6*
	10	--	--	--	--	--	--	--	7.4	7.4
	20	6.3	60/45	60/45	29.4	70/53	--	27.6	--	--
	25	--	--	--	--	--	--	--	7.4	5.4*
	35	16.3	95/65	95/64	29.4	72/55	--	29.4	--	--
	40	--	--	--	--	--	--	--	7.7*	8.0*
	50	3.0	80/60	82/60	33.1	75/55	--	27.9	--	--
	55	--	--	--	--	--	--	--	7.4	7.8*
	72	--	--	--	--	--	--	--	--	4.5*
	80	--	--	190/130	--	--	--	--	--	--
45	0	0	87/37	74/43	26.5	60/42	14.7	25.7	7.3	7.4
	3	0	79/39	70/40	26.5	59/42	--	22.8	--	--
	11	--	--	--	--	--	--	--	7.2*	6.5*
	20	0	75/30	64/32	14.7	61/45	--	25.0	--	--
	25	--	--	--	--	--	--	--	6.5*	6.8*
	35	0	88/32	69/32	23.5	58/48	19.9	26.1	--	--
	50	0	86/36	78/38	23.5	58/49	--	27.2	--	--
	55	--	--	--	--	--	--	--	7.4*	3.5*
	65	3.0	89/45	82/45	22.8	64/50	--	27.6	--	--
	70	--	--	--	--	--	--	--	8.3*	3.5*
	85	20.0	135/85	135/85	25.7	59/50	--	27.2	--	--
	93	--	--	--	--	--	--	--	--	9.5*
	105	24.0	125/74	130/50	--	--	--	--	--	--
	115	18.5	135/75	120/80	18.4	65/43	--	27.9	--	--

* pH readjusted to 7.4 by dropwise addition of 1N HCl or 1N NaOH

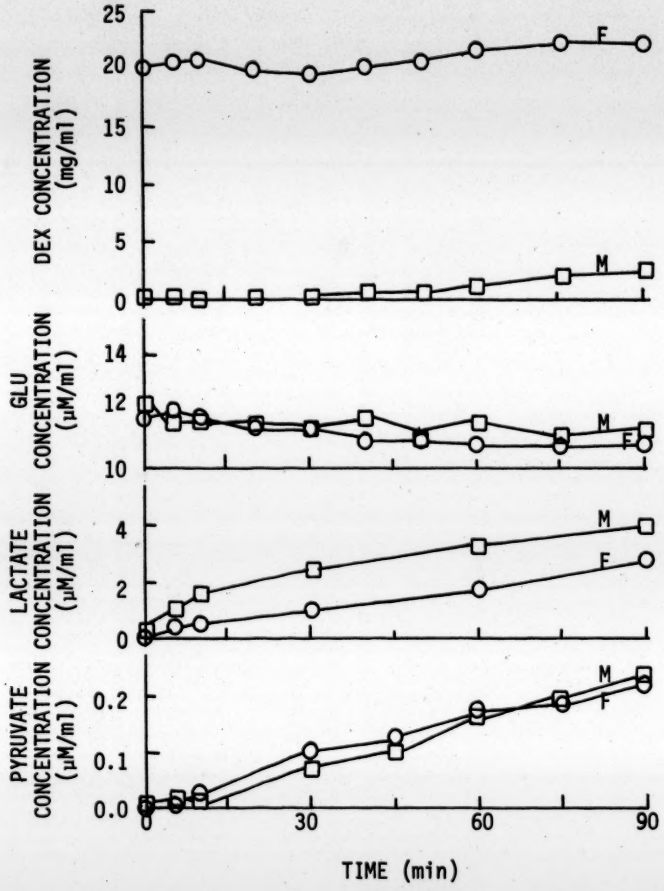


Figure 24. Concentrations of DEX, GLU, lactate and pyruvate in fetal reservoir perfusate (F) and maternal reservoir perfusate (M) during perfusion of placenta 42.

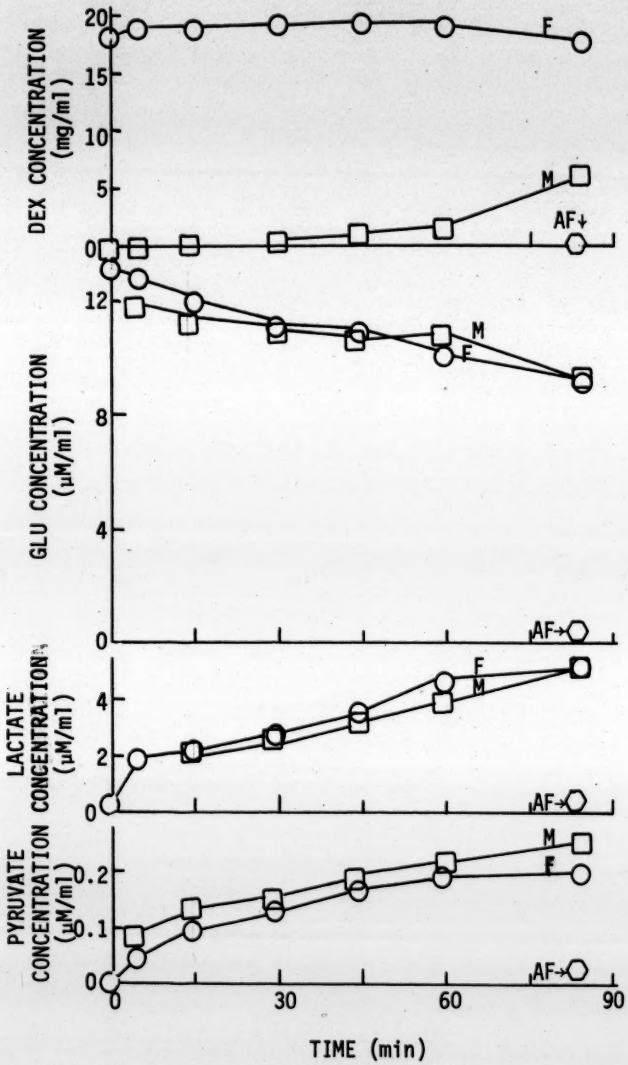


Figure 25. Concentrations of DEX, GLU, lactate and pyruvate in fetal reservoir perfusate (F), maternal reservoir perfusate (M), and amniotic chamber fluid (AF) during perfusion of placenta 43.

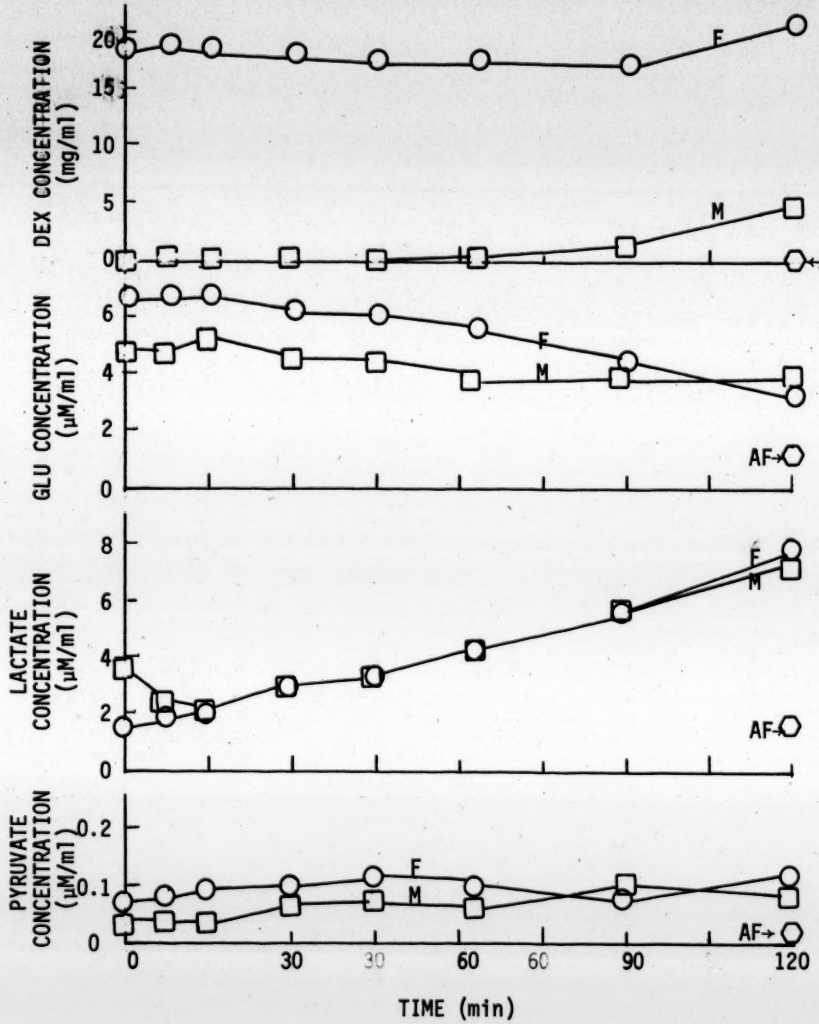


Figure 26. Concentrations of DEX, GLU, lactate and pyruvate in fetal reservoir perfusate (F), maternal reservoir perfusate (M), and amniotic chamber fluid (AF) during perfusion of placenta 45.

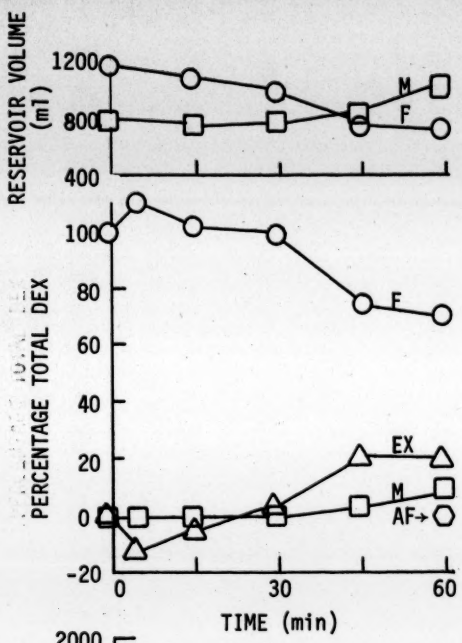


Figure 27. Fetal and maternal reservoir volumes and percentages of total DEX accounted for in the fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF) and amounts not accounted for in perfusates (EX) during perfusion of placenta 43.

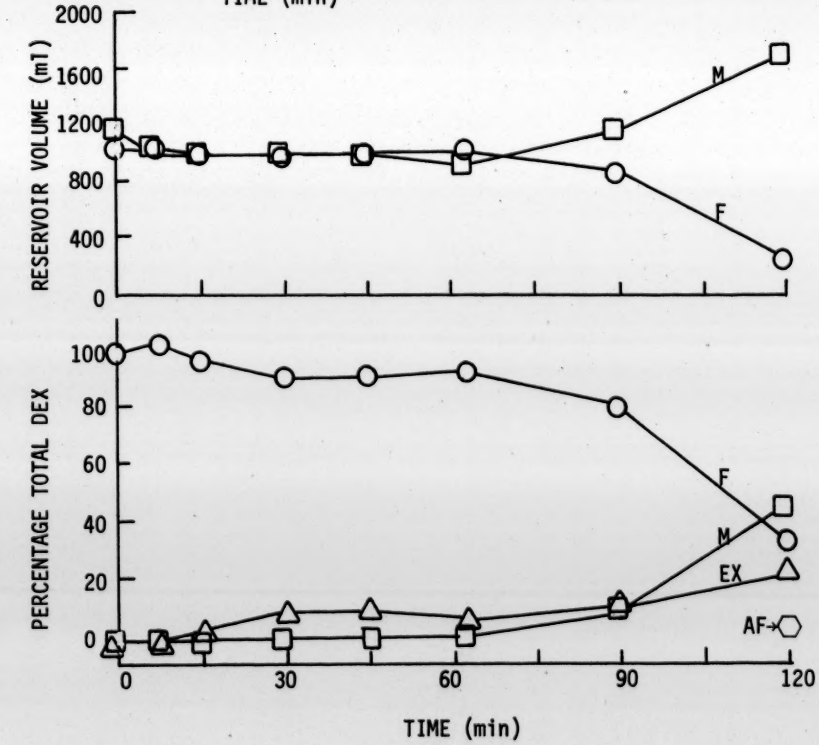


Figure 28. Fetal and maternal reservoir volumes and percentages of total DEX accounted for in the fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF), and amounts not accounted for during perfusion of placenta 45.

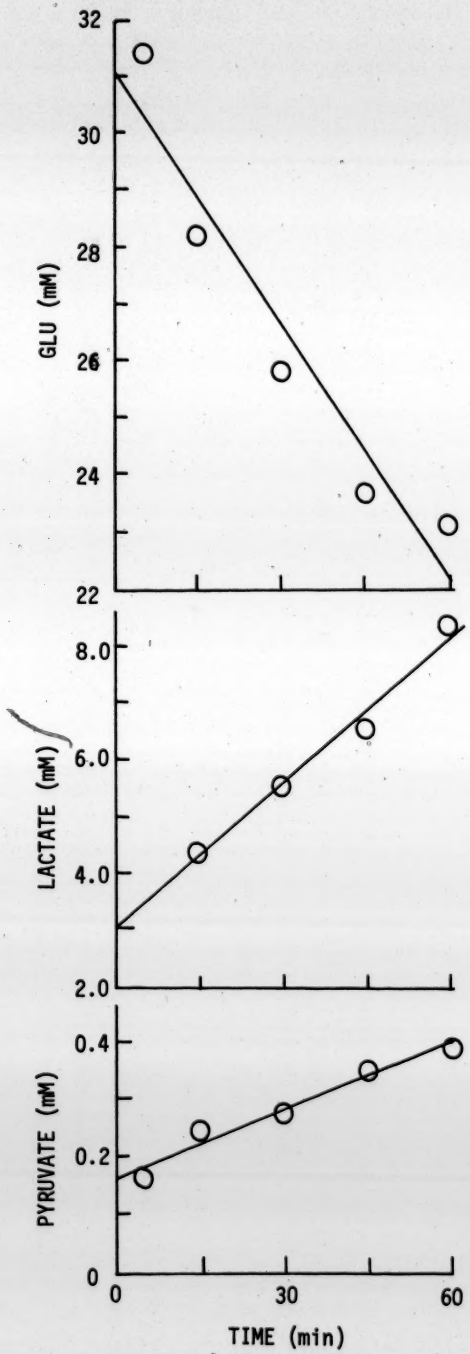


Figure 29. Total amounts of GLU, lactate and pyruvate accounted for in perfusates during perfusion of placenta 43, and calculated linear regression lines.

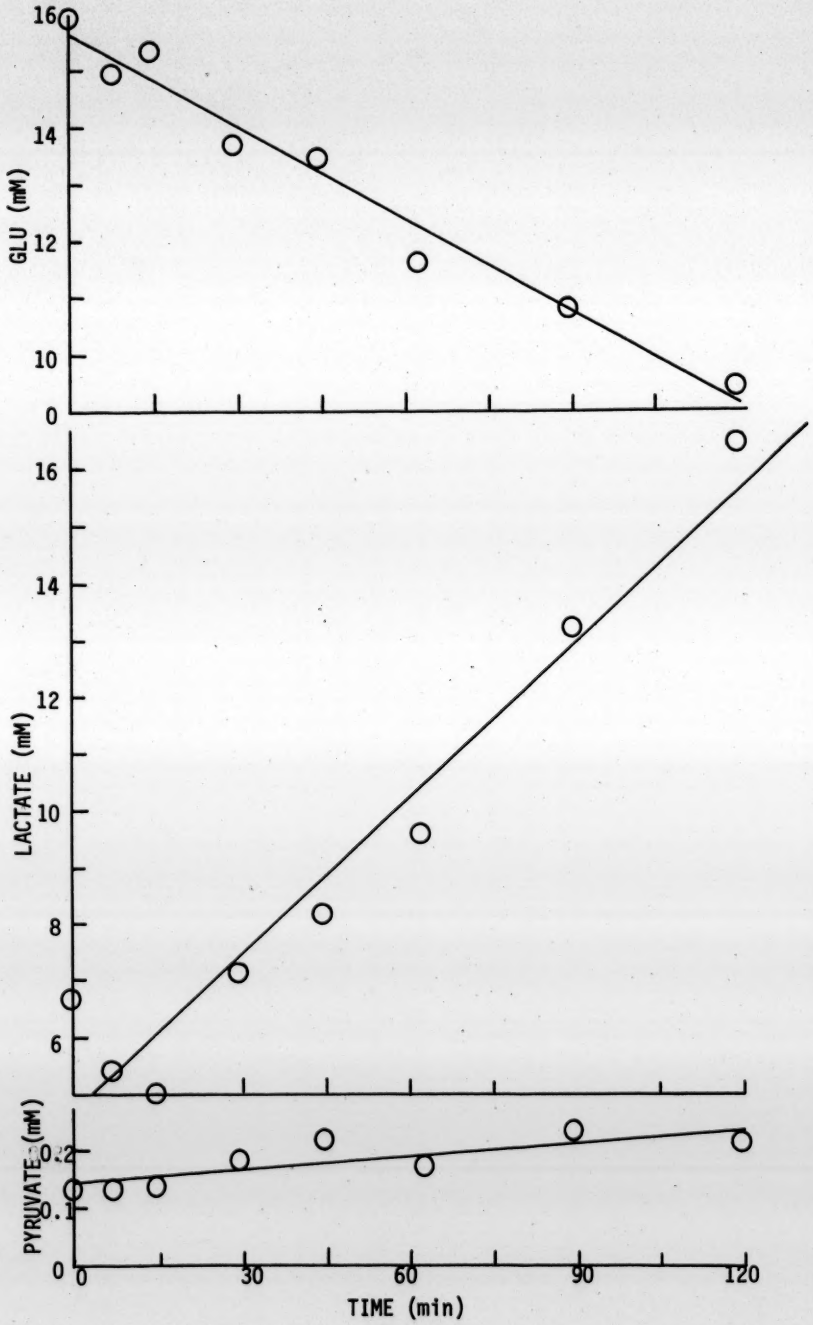


Figure 30. Total amounts of GLU, lactate and pyruvate accounted for in perfusates during perfusion of placenta 45, and calculated linear regression lines.

The addition of low molecular weight dextran (DEX) to the fetal perfusates may have been successful in opposing the small rates of fetal to maternal fluid flow expected when fetal arterial pressures were in the in vivo range and no mechanical or hypoxic damage to the capillaries was encountered.

Disposition of low molecular weight dextran (DEX).--DEX concentration in fetal perfusates remained relatively constant during the perfusions, except for a slight tendency for fetal DEX concentrations to increase during periods in which there was rapid fetal to maternal fluid flow (figures 24-26). In this respect, there is some resemblance to the disposition of BD as observed previously.

On the other hand, unlike BD, about 10% of the DEX initially added to the fetal perfusate at the start of the experiment with placenta 45, left the fetal circuit within the first thirty minutes of perfusion, during which time there was no measurable loss of fluid from the fetal circuit (figure 28). No further loss of DEX from the fetal circuit occurred until the start of fetal to maternal fluid flow, after the first hour of perfusion. DEX was continuously lost from the fetal circuit of placenta 43 in which there was also continuous transfer of fluid from the fetal to the maternal circuit (figure 27).

Reports of the placental transfer of various dextran fractions in vivo have been conflicting. The inability to detect dextran in the cord blood of infants whose mothers were infused with low molecular weight dextran during labor, was reported in two separate studies (174-175). Maternal serum concentrations of dextran were as high as 1 g/100 ml in one of

those studies (175). On the other hand, another report (176) states that dextran was detected in the cord blood of infants whose mothers were infused during labor with solutions of a higher molecular weight dextran preparation (average molecular weight 70,000 with 90% of the molecules having molecular weights between 50,000 and 100,000). Dextran plasma levels of mothers whose neonates had detectable amounts of dextran in their cord blood, ranged from 0.25-0.5 g/100 ml.

Labeling information supplied by the manufacturer indicates that low molecular weight dextran (DEX) is a mixture of glucose polymers whose average molecular weight is about 40,000. About 10% of the polymers have molecular weights below 18,000 and 10% above 82,000.

It is probable that the low molecular weight polymers of this mixture are capable of permeating intact capillaries with little restriction. If the percentage of the more permeable fraction of low molecular weight dextran molecules is 10% as suggested by the experiment with placenta 45, the amount of dextran which crosses the placenta in vivo would be less than 1% of the dose since the placenta receives less than 10% of the maternal cardiac output (58). Thus, the conflicting in vivo reports may reflect differences in sensitivity of the methods employed to determine dextran concentrations.

The remaining 90% of dextran molecules which are restricted in their permeability to intact fetal capillaries, leave the fetal circuit only upon damage, as evidenced by rapid fetal to maternal fluid flow and high fetal arterial pressures.

Thus, DEX may also be used as a suitable indicator to

monitor bulk leakage of perfusate from fetal to maternal circuit, since only 10% leaves the intact fetal circuit.

Most of the DEX which left the fetal circuit initially, is not accounted for in the maternal perfusate. DEX was detected in maternal perfusate only after a 30-60 minute lag period (figures 24-28). This may be indicative of restricted diffusion within the placental tissues.

Only a small proportion of the DEX which was not accounted for in maternal or fetal perfusates at the conclusions of the experiments were detected in the samples collected from the amniotic fluid chamber. The percentages of DEX detected in the amniotic chamber fluid samples were 0.076% and 3.24% for placentas 43 and 45, respectively. The high percentage of DEX in the amniotic fluid after perfusion of placenta 45 may be due to direct contact of the fluids in the placental and amniotic chambers during perfusion of that placenta. It was noted at the conclusion of the experiment that the fetal membranes had not been completely pulled over the edge of the placental chamber of the apparatus. High percentages of GLU, lactate and pyruvate were also found in the amniotic fluid sample from placenta 45.

Disappearance of dextrose (glucose, GLU).--At the start of each experiment, concentrations of GLU in fetal perfusate were about equal to or slightly higher than GLU concentrations in maternal perfusates (figures 24-26). During each experiment, GLU concentrations in both maternal and fetal perfusates declined with time and a maternal to fetal GLU concentration gradient was established.

The establishment of a maternal to fetal concentration

gradient of GLU conforms with results reported by other investigators perfusing the placenta ex vivo (123,125,138), and with in vivo measurements (23). The possible significance of this gradient with respect to transfer mechanisms will be discussed in a later part of this work.

Figures 29 and 30 show total amounts of GLU accounted for in the maternal and fetal perfusates during perfusions of placentas 43 and 45. The lines drawn in those figures are the linear regression lines calculated from the data by the method of least squares. The slopes of the linear regression lines represent the negative value of the average disappearance rates of GLU from the perfusates during the experiments. The average disappearance rates for GLU during perfusion of placentas 43 and 45 were respectively 14.78 and 5.88 micromoles GLU per gram wet placental weight per hour. Table VII compares the rates found in this study with glucose disappearance rates found by other investigators during ex vivo placental perfusion or in vitro incubation of placental tissue studies. The average rates of glucose disappearance reported in this study are within the range of values reported by other investigators.

The significance which can be placed upon the value calculated as the average disappearance rate of GLU is limited. It is simply a qualitative indicator of the metabolic ability of the tissues. The reasons for this limitation are as follows:

1) The disappearance rate of GLU from the medium during perfusion is the algebraic sum of the GLU utilization rate of the placenta and the net diffusion rate between the perfusate and placental tissues. Thus, as Nesbitt, et al. (139), point

TABLE VII

Studies of Glucose Disappearance During Placental Perfusion and Incubation of Placental Tissues Reported in the Literature

TYPE OF STUDY	RATE OF GLUCOSE DISAPPEARANCE	REMARKS	REFERENCE
	µM/g/hr*		
Ex-vivo Placental Perfusion	5.88-14.78	---	This study
	5.56	Constant over 12 hours	Goerke, et al. (177)
	6.67	---	Krantz, et al. (123)
	6.94	---	Howard and Krantz (127)
	6.4	---	Ray and Krantz (125)
	3.33-16.7	---	Nesbitt, et al. (138)
	7.72-8.83	Steady-state Conditions	Nesbitt, et al. (138)
	2.9-5.09	---	Krantz, et al. (134)
In-vitro Incubation of placental tissue slices	8.0	---	Hagerman and Villee (178)
	7.0	---	Villee (179)
	2.59-5.05	---	Krantz, et al. (134)
	0 - 2.5	2.8 mM/l Glucose	Szabo, et al. (180)
	1.39 - 5.83	5.6 mM/l Glucose	
	6.39 - 13.3	16.7 mM/l Glucose	

*micromoles of glucose per gram wet weight of placenta per hour

out, the rate of GLU disappearance from the perfusate is an overestimate or underestimate of GLU utilization by the tissues, depending on the direction of net transfer between perfusate and tissues. Maintenance of a constant GLU concentration in perfusates (steady state) is necessary in order to quantitate GLU utilization rates. Nesbitt's studies show that GLU disappearance from the perfusate under non steady state conditions ranged from 3.3 to 16.7 micromoles GLU/gram wet placental weight/hr, and 7.72-8.33 uM/g/hr under steady state conditions.

2) The rate of GLU disappearance has been shown to be dependent on initial GLU concentrations in perfusate or incubation medium (134,138,180).

3) The rate of GLU disappearance may be dependent on the availability of oxygen to the placenta; however, results of studies are conflicting. Some investigators have shown the GLU disappearance rate to increase with increasing oxygen tension in the perfusate or incubation medium (138,181), while others have shown no significant difference in GLU utilization rates when placental tissues are incubated in atmospheres of 100% oxygen or 100% nitrogen (182).

4) Utilization of GLU by individual placentas may vary according to the glycogen content of the placental tissues. Villee has pointed out the ability of the placental tissue slices to utilize stored glycogen during incubation at rates equivalent to 1.92 micromoles GLU/g wet weight placenta/hr (179).

5) Finally, the time between delivery of placenta and initiation of perfusion may affect the rate of GLU disappearance. Villee has demonstrated that a delay of 60 minutes between

delivery of the placenta and incubation of placental tissue slices results in a 20% decrease in GLU disappearance rates compared to rates measured when tissue slices from placentas are incubated within 5 minutes of delivery (179). The time between delivery of the placenta and initiation of the perfusion in these experiments varied from 35 to 60 minutes. Other investigators, listed in table VII, reported intervals of 10-30 minutes between delivery of the placenta and initiation of perfusion.

Appearance of lactate.--Lactate was detected in the first samples collected from maternal and fetal perfusates during each experiment. Concentrations of lactate in both perfusates increased with time of perfusion (figures 24-26). Lactate was also detected in samples of fluid collected from the amniotic fluid chamber after perfusing placentas 43 and 45 (figures 25, 26).

The total amounts of lactate found in both perfusates during perfusion of placentas 43 and 45 are plotted against time in figures 29 and 30. The average rates of appearance of lactate during each of these experiments, calculated from the slopes of the least squares linear regression lines shown in figures 29 and 30, were 8.47 and 10.02 micromoles lactate per gram wet placental weight per hour for placentas 43 and 45, respectively.

Ray and Krantz (125) reported lactate appearance rates averaging 11.3 micromoles lactate/g wet weight placenta/hr during ex vivo perfusion of the human placenta. Villee (179) reported lactate appearance rates ranging from 9.7 to 13.1 micromoles/g/hr for placental tissue slices incubated with GLU

under an oxygen atmosphere, and rates ranging from 21.1 to 23.3 for slices incubated with GLU under an atmosphere of 100% nitrogen. Villee also noted a 20% decrease in lactate appearance rates found when there was a 60 minute interval between delivery of the placenta and initiation of incubation with GLU under an oxygen atmosphere, compared to rates found with only ten minutes between delivery and incubation under the same conditions.

Thus, the results reported in this study are consistent with those reported in the literature for placental tissues utilizing glucose as a nutrient under aerobic conditions.

Appearance of pyruvate.--Like lactate, pyruvate was also detected in the first samples collected from both maternal and fetal perfusates and likewise, concentrations of pyruvate increased with time during perfusion of placentas 42, 43, and 45 (figures 24-26).

Total amounts of pyruvate found in both perfusates during perfusion of placentas 43 and 45 are plotted in figures 29 and 30. The average rates of appearance of pyruvate during these experiments, calculated from the slopes of the least squares regression lines, were 0.435 and 0.0826 micromoles pyruvate per gram wet placental weight per hour for placentas 43 and 45, respectively.

In vitro incubation studies with placental tissues show that because pyruvate is produced from glucose metabolism and also used by the tissues as a nutrient, the net result of incubation may be disappearance or appearance, depending on the composition of the incubation medium (179). For example, when

placental tissues were incubated in a medium containing in each liter, 10 mM pyruvate and 11.1 mM glucose, there was net utilization of pyruvate. It was demonstrated in these studies that the net utilization rate of 8.3 uM/g/hr was the algebraic sum of utilization of added pyruvate at the rate of 22.3 uM/g/hr, and the production at the rate of 14 uM/g/hr, 5 uM/g/hr of which was produced from the added glucose.

Rates of pyruvate appearance or disappearance during ex vivo perfusion of the human placenta have not previously been reported. Concentrations of pyruvate in perfusates during ex vivo perfusions of the placenta published in two studies in which glucose was present in the perfusates, showed a net increase in pyruvate with time (125,134). Concentrations of pyruvate during perfusion, published in another study, in which no nutrient was added to the perfusion medium, showed a net disappearance in pyruvate which was contained in the blood used for perfusion medium (134).

The results shown in this study, therefore, are consistent with those of a placenta utilizing glucose as a nutrient.

Perfusate pH.--The range of pH values measured in these experiments was 3.5 to 9.5, table VI. There was, in general, a tendency for pH to decline with time in both perfusates. However, an occasional high pH measurement was observed.

A fall in pH during the perfusion was expected because of production of lactic acid, CO_2, and other organic acids produced in the course of normal cellular metabolism. The decrease in pH with time was more rapid in these experiments than in those reported by Goerke and co-workers (177). Figure 3 in

their paper shows a linear decrease in pH from 7.6 at the start of perfusion to 6.5 after twelve hours of perfusion. The same perfusate was circulated through the umbilical vessels as well as through the bath which was in contact with the decidual side of the placenta. There was no perfusion of the intervillous space. The perfusate which they used contained a multitude of amino acids as well as albumin, all of which may have provided a high buffer capacity to the perfusate, thereby preventing rapid changes in pH.

Viability of placentas.--In a recent review on cell death in isolated perfused organs, Cohen and Folkman (167) divided the steps toward death taken by an injured cell into three phases: Phase 1) a period of reversible deterioration in which injury is done to the cell but the damage is not so extensive that recovery is impossible, Phase 2) the period of dying in which the cell sustains sufficient injury to pass the "point of no return," and Phase 3) the period of death in which cellular organization is disrupted and integrated cellular functions cease. They state that the speed with which different cells will move through these stages depends on the innate characteristics of the cell as well as the stresses to which the cells are subjected.

The results presented for these experiments show that the placenta as a whole when perfused, ex vivo, with a protein-free perfusate, similar in electrolyte composition to plasma, containing dextrose as a nutrient, and provided with oxygen, is capable of metabolic activity. Indications of metabolic activity are the disappearance of GLU from the perfusates with

the appearances of lactate, pyruvate, and hydrogen ion.

The lack of viability, of at least some of the cells of the perfused placenta, is shown by 1) the development of increased fetal perfusion pressures concurrent with fetal to maternal fluid flow at rates greater than 2 ml/min, and 2) increased permeability of the placenta to high molecular weight fractions of dextran and to BD. Differences in the susceptibility of various placental tissues to hypoxic conditions have been demonstrated by several investigators.

Panigel (183) has shown, by means of electron microscope examination of placental villi after perfusion of fetal capillaries and intervillous space of the human placenta, that the syncytiotrophoblast cells are the most susceptible to cell death ex vivo. When perfusion is performed for two hours with inadequately oxygenated medium, extensive changes occur in the syncytiotrophoblast with little or no change in other layers. Such changes include: 1) reduction in the number of microvilli, 2) appearance of clear spaces between the syncytiotrophoblast and the cytotrophoblast, and within the syncytiotrophoblast itself, and 3) dilatation of the endoplasmic reticulum within the syncytiotrophoblast.

This higher susceptibility of the syncytiotrophoblast to hypoxia has been substantiated by studies in which incubation of placental tissue slices is carried out in an atmosphere of 6% oxygen (184-185). Tominaga and Page (185) further reported that the thinning of the syncytiotrophoblast which occurs after six hours of incubating placental villi in a 6% oxygen atmosphere is reversible if oxygen content is increased to 20% after six

hours, but is irreversible after twelve hours in 6% oxygen atmosphere.

One possible explanation for the loss of viability of placenta tissues after initiation of perfusion is that inadequate supplies of nutrient and oxygen are supplied to the organ. At least 15 mM of GLU were available in the perfusates at the start of the experiments. This amount of GLU is sufficient for three hours utilization by a 500 g placenta at rates up to 10 uM/g/hr. Goerke, et al. (177), have shown that saturation of maternal perfusate with oxygen would be adequate to provide the needs of the placenta. Figures provided in their paper show that a flow rate to the placenta of 74 ml/min of such an oxygenated perfusate would supply the needs for a 500 g placenta. Maternal perfusate flow rates in the study were more than 8 times this minimum figure, assuring a more than adequate supply of oxygen.

A second, and more likely reason for cell death after initiation of perfusion is that the cells most susceptible to ischemia have passed into the phase of irreversible injury before initiation of perfusion. The placentas used in this study were subject to at least two periods of ischemia.

The first period of ischemia is that which occurs during labor. All of the placentas used for this study were obtained after normal term deliveries. During labor, the maternal blood supply to the placenta is impeded while the uterus is contracting (163). The duration of uterine contractions and their frequency vary a great deal among women in labor. Since the placenta derives oxygen and nutrients from the maternal blood,

periods of uterine contraction represent ischemic periods for the placenta. It is not known how much, if any, irreversible cell death occurs among placental cells during labor.

The second period of ischemia occurs from the time separation of the placenta from the uterus takes place, until ex vivo perfusion of the maternal circuit with an adequate supply of oxygen and nutrients is begun. In this study, the duration of time between delivery of the placenta, which takes place minutes after placental separation from the uterus, and start of the perfusion ranged from 35-60 minutes. It was not possible to reduce this time during the study due to a transit time from delivery room to laboratory of from 5 to 10 minutes, and the availability of only one pair of hands to prepare, cannulate, and mount the placenta in the apparatus. The report by Villee (179) that a twenty percent decrease in the glucose of disappearance rates and in the rates of appearance of lactate and pyruvate occur for placental tissue slices incubated 60 minutes after delivery of the placenta, compared to rates measured for slices which are incubated 10 minutes after delivery, may be indicative of the amount of cell death which takes place during this ischemic period.

Thus, the prospects of improving the viability of the placentas, thereby avoiding the occurrence of rapid maternal to fetal fluid flow, look dim.

For the purposes of the experiments, viability is defined as maintenance of membrane integrity. Viability may also be monitored by the measurement of some metabolic function of the placenta such as HCG production rate or steroid conver-

sions. However, because these experiments are concerned with the transfer function of the placenta, rather than the metabolic function, viability may more appropriately be monitored simply by monitoring fetal to maternal fluid flow and permeability to DEX.

Conclusions

The conclusions drawn from these experiments may be summarized as follows:

1. The apparatus used to perfuse the human placenta ex vivo is of such a design as to make possible separation of the three fluid compartments with which the placenta comes into contact: 1) maternal perfusate, 2) fetal perfusate, and 3) amniotic fluid.

2. Poor availability of placentas suitable for perfusion severely limited the scope of this research.

3. Fetal perfusate flow rates must be maintained at values less than one-third the value believed to exist in vivo. Amniotic fluid pressure required is greater than the pressure in vivo when the uterus is at rest, but lower than the in vivo amniotic fluid pressures which exist while the uterus is contracting. The other flows and pressures maintained during ex vivo perfusion are comparable with in vivo values.

4. Flow of fluid from the fetal to the maternal circuit at rates less than 2 ml/min with retention in the fetal circuit of BD or of 90% of DEX is to be expected due to a normal fetal to maternal hydrostatic pressure gradient.

5. Flow of fluid from the fetal to maternal circuit at rates greater than 2 ml/min is usually accompanied by increased

fetal perfusion pressures and passage of BD or more than 10% DEX from the fetal to the maternal circuit. Such circumstances are indicative of reduced viability of the most susceptible cells of the placenta caused by hypoxia and/or lack of globulins in the perfusate.

6. The placenta as a whole is capable of aerobic metabolism of glucose even in the face of the circumstances described above as indicators of partial viability loss.

7. The placenta is permeable to BSP as evidenced by the appearance of the compound in the maternal circuit after its rapid disappearance from the fetal circuit. Most of the BSP which leaves the fetal circuit is not accounted for in the maternal circuit. It is possible that BSP is highly bound to placental tissue proteins.

8. Transfer of ANT across the placenta in either direction is rapid, even when opposed by high fetal to maternal fluid flow.

9. The employment of this ex vivo perfusion method to study factors affecting the disposition of drugs across the human placenta, is limited by the period of time in which placentas exhibit characteristics of viability.

DISPOSITION OF SUBSTANCES IN THE EX VIVO PERFUSED HUMAN PLACENTA

With the limitations imposed by the low availability of placentas suitable for perfusion, and by the unpredictable period in which perfused placentas showed evidence of viability, it became necessary to design experiments for the study of drug disposition in which maximum data could be obtained from few experiments. Therefore, as many of the drugs as could be simultaneously assayed were added to either maternal or fetal perfusate at the start of each experiment. This design allowed for comparisons to be made among the test substances of the initial rates of disappearances from the perfusate to which the substances were simultaneously added, rates of appearance in the opposite reservoir, amounts transferred to the amniotic chamber fluid, and amounts of each substance not accounted for in perfusates and in amniotic chamber fluid. Dextrose (GLU), which had to be supplied in the perfusate as a nutrient for the placenta, was always added with the drugs so that comparisons could be made between the disposition of GLU and the disposition of the drugs. Placental viability could be monitored as discussed in the previous section.

In order to determine the extent to which the fraction of each substance was taken up by or destroyed in the presence of the plastic components of the apparatus, perfusions of both fetal and maternal circuits of the apparatus without a placenta (blank perfusions) were performed.

Finally, partition coefficients of each substance between octanol and the perfusion solution used in these

experiments, was determined in order to test correlation of this parameter with each of the parameters of disposition studied.

Experiments

Placental Perfusions

The placentas were perfused with electrolyte perfusates which were oxygenated as described in the Materials and Methods section. The substances added to each perfusate and the fluids sampled during each experiment are listed in table VIII.

TABLE VIII

Disposition Experiments

Placenta	Substance Added to		Fluids Sampled
	Maternal Perfusate	Fetal Perfusate	
47	ANT*,SNL,ASL,GLU	DEX	MA,FA,AF
50	ANT,SNL,ASL,BSP,GLU	DEX	MA,MV,FA, FV,AF
51	ANT,SDM,ASL,BSP,GLU	DEX	MA,MV,FA, FV,AF
54	-----	ANT,SDM,ASL,BSP, GLU,DEX	MA,MV,FA, FV,AF
55	-----	ANT,SDM,ASL,BSP, GLU,DEX	MA,MV,FA, FV,AF

*ANT = antipyrine, SNL = sulfanilamide, ASL = N-4-acetylsulfanilamide, SDM = sulfadimethoxine, BSP = sulfobromophthalein, GLU = dextrose, DEX = low molecular weight dextran, MA = maternal reservoir perfusate, MV = maternal venous perfusate, FA = fetal reservoir perfusate, FV = fetal venous perfusate, AF = amniotic chamber fluid.

Perfusion procedures, volume measurements, sampling procedures, deproteination of samples, and assay procedures were carried out as described in the Materials and Methods section.

Fetal Blank Perfusions

The fetal arterial cannulas were connected through a "y-tube" to the venous cannula and the fetal circuit primed with 106.0 ml heparinized Ringer's solution, warmed to 38°. One liter of electrolyte perfusate, to which had been added 1 mM each ANT, SDM, ASL, and BSP, 10 mM GLU, and 20 g DEX, was warmed to 38°, pH adjusted to 7.4, and filtered through Whatman #1 filter paper. The fetal reservoir was filled with the warmed perfusion solution, a 2.0 ml sample withdrawn, and the pump was turned on. Further samples were collected from the fetal reservoir 2, 5, 10, 15, 60, 120, and 180 minutes after beginning the perfusion. All samples were immediately deproteinated, centrifuged, and refrigerated until ready for assay for ANT, SDM, ASL, BSP, GLU, and DEX.

Maternal Blank Perfusions

The maternal circuit of the apparatus was primed with 456.0 ml heparinized Ringer's solution warmed to 38°. The rim of the placental chamber was coated with silicon stopcock lubricant (Dow Chemical Co.) and the chamber was covered with a 12" square glass plate (pharmaceutical ointment slab). The slab was clamped on four sides to the placental chamber with "c-clamps." A 1500 ml volume of electrolyte perfusion solution, to which had been added 1.5 mM each ANT, SDM, ASL, and BSP, 15 mM GLU, and 3.0 g DEX, was warmed to 38°, pH adjusted to 7.4, and filtered

through Whatman #1 filter paper. The maternal reservoir was filled with the warmed filtered perfusion solution, a 2.0 ml sample collected, the pump turned on, and further samples withdrawn and handled as with the fetal blank perfusion.

Partition Coefficients

Solutions of the following substances and concentrations were prepared in electrolyte perfusate and warmed to 37°; 1 mM/l ANT, 1 mM/l SNL, 1 mM/l SDM, 1 mM/l ASL, 1 mM/l BSP, 10 mM/l GLU, and 20 mg/ml DEX. The pH of each solution was adjusted to 7.4 by the addition of a few drops of 1 N HCl. The solution of SDM was filtered after adjustment of the pH to remove any undissolved drug. The solutions thus prepared were used to determine partition coefficient by the method described in the Materials and Methods section.

Results and Discussion

Viability of Perfused Placentas

The reservoir volumes measured during each of the perfusion experiments are listed in tables IX-XIII and plotted at the times of measurement in figure 31. It can be seen by examination of the data that fetal to maternal flow rates greater than 2 ml/min were encountered at the start of perfusing placentas 47 and 55, after 10 minutes of perfusing placenta 50, after 80 minutes of perfusing placenta 51, and after 40 minutes of perfusing placenta 54.

The concentrations of DEX measured in samples collected during the experiments are listed in tables IX-XIII and are plotted, as ratios of the zero time fetal reservoir DEX

TABLE IX

Volumes Measured and Concentrations of Substances Determined in Samples of Fluids from Placenta 47.

TIME	FLUID	VOLUME	CONCENTRATIONS				
			DEX	GLU	ANT	SNL	ASL
min		ml	mg/ml	µM/ml	µM/ml	µM/ml	µM/ml
0	M	--	4.26	13.85	0.706	0.658	0.706
	F	--	8.18	00.000	0.0000	0.0000	0.0000
	AF	--	--	--	--	--	--
5	M	2000	4.58	11.15	0.574	0.542	0.596
	F	650	7.16	0.000	0.0250	0.0102	0.0055
10	M	2150	4.87	11.43	0.534	0.509	0.552
	F	500	6.69	0.337	0.0632	0.0234	0.0177
15	M	2200	4.87	10.12	0.506	0.502	0.520
	F	375	7.13	0.588	0.1072	0.0415	0.0293
20	M	2200	5.06	9.60	0.498	0.480	0.534
	F	375	7.56	0.879	0.1596	0.0674	0.0512
25	M	2200	5.27	9.41	0.474	0.477	0.527
	F	275	7.18	1.20	0.200	0.0885	0.0726
30	M	2300	5.19	8.93	0.456	0.455	0.501
	F	225	7.76	1.65	0.2452	0.1169	0.0890
	AF	925	2.69	0.595	0.0159	0.0078	0.0073

KEY FOR TABLES IX-XIII: Maternal reservoir perfusate (M), Fetal reservoir perfusate (F), Maternal venous perfusate (MV), Fetal venous perfusate (FV), Amniotic chamber fluid (AF), not determined (--).

TABLE X

Volumes Measured and Concentrations of Substances Determined in Samples of Fluids from Placenta 50.

TIME	FLUID	VOLUME	CONCENTRATIONS					
			DEX	GLU	ANT	SNL	ASL	BSP
min		ml	mg/ml	µM/ml	µM/ml	µM/ml	µM/ml	µM/ml
0	M	1200	0.00	8.259	1.060	0.9196	0.8909	0.8743
	MV	--	0.00	0.000	0.0000	0.0000	0.0000	0.0000
	F	950	19.44	0.000	0.0000	0.0000	0.0000	0.0000
	FV	--	0.00	0.000	0.0000	0.0000	0.0000	0.0000
	AF	--	0.00	0.000	0.0000	0.0000	0.0000	0.0000
2	M	1200	0.00	8.222	1.005	0.8869	0.8744	0.8727
	MV	--	0.00	7.193	0.8462	0.7890	0.7812	0.7631
	F	950	16.87	0.474	0.0008	0.0006	0.0000	0.0005
	FV	--	16.17	0.323	0.0410	0.0181	0.0110	0.0022
5	M	1200	0.00	8.102	0.9325	0.8471	0.8269	0.8181
	MV	--	0.00	8.062	0.6762	0.8047	0.7830	0.5901
	F	950	17.02	0.193	0.0129	0.0079	0.0024	0.0010
	FV	--	17.42	0.460	0.0439	0.0309	0.0195	0.0035
10	M	1265	0.25	7.548	0.8735	0.8035	0.7830	0.7732
	MV	--	0.39	7.112	0.8054	0.7564	0.7702	0.7089
	F	940	17.08	0.285	0.0295	0.0224	0.0116	0.0024
	FV	--	17.05	0.449	0.0658	0.0484	0.0384	0.0057
15	M	1295	0.44	7.112	0.8049	0.7893	0.7373	0.7270
	MV	--	0.65	5.925	0.8109	0.6941	0.6238	0.6367
	F	820	17.74	0.327	0.0545	0.0344	0.0195	0.0043
	FV	--	12.57	0.424	0.0644	0.0393	0.0433	0.0044
20	M	1325	0.80	6.833	0.7869	0.7638	0.6696	0.6663
	MV	--	0.91	6.911	0.7688	0.7493	0.6732	0.6538
	F	750	16.39	0.368	0.0566	0.0503	0.0256	0.0061
	FV	--	17.19	0.514	0.0862	0.0654	0.0506	0.0081
30	M	1405	1.42	6.671	0.7425	0.6653	0.5964	0.6121
	MV	--	1.55	6.157	0.6425	0.6073	0.6001	0.5681
	F	655	17.48	0.539	0.0938	0.0720	0.0524	0.0099
	FV	--	15.91	0.542	0.1104	0.0841	0.0622	0.0102
40	M	1485	1.93	6.154	0.6013	0.5966	0.5854	0.5195
	MV	--	2.08	6.865	0.6520	0.5816	0.6000	0.5335
	F	535	19.33	0.692	0.1235	0.0956	0.0799	0.0125
	FV	--	16.87	0.622	0.1302	0.1084	0.0805	0.0127
50	M	1540	2.52	5.913	0.5788	0.5674	0.5561	0.5174
	MV	--	2.98	6.108	0.5974	0.5490	0.5561	0.4882
	F	440	19.11	0.661	0.1534	0.1229	0.0939	0.0146
	FV	--	18.37	0.632	0.1579	0.1302	0.0948	0.0156
60	M	1645	3.14	6.028	0.5857	0.5783	0.5232	0.4738
	MV	--	2.83	5.832	0.5609	0.5383	0.5342	0.4502
	F	345	18.95	0.688	0.1913	0.1446	0.1104	0.0167
	FV	--	18.59	0.644	0.1935	0.1501	0.1223	0.0178
70	M	1725	3.00	4.843	0.5112	0.4873	0.4464	0.4329
	MV	--	3.60	5.116	0.5476	0.5091	0.5049	0.4076
	F	250	19.78	0.657	0.2269	0.1713	0.1329	0.1204
	FV	--	19.48	0.665	0.2107	0.1781	0.1418	0.0209
80	M	1805	4.46	4.953	0.5246	0.5092	0.4903	0.4154
	MV	--	4.16	5.034	0.5205	0.4763	0.4830	0.3755
	F	205	20.61	0.632	0.2228	0.1829	0.2037	0.0221
	FV	--	20.32	0.568	0.2432	0.1926	0.1597	0.0229
	AF	1400	0.003	0.026	0.0044	0.0103	0.0037	0.0000

TABLE XI

Volumes Measured and Concentrations of Substances Measured in Samples of Fluids from Placenta 51

TIME	FLUID	VOLUME	DEX	GLU	ANT	SDM	ASL	BSP
min		ml	mg/ml	μM/ml	μM/ml	μM/ml	μM/ml	μM/ml
0	M	1500	0.000	5.218	0.9561	0.6544	1.0737	0.9295
	MV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	F	1000	19.54	0.0000	0.0000	0.0000	0.0000	0.0000
	FV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	AF	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
2	M	1550	0.000	4.328	0.8442	0.6198	0.9366	0.8240
	MV	--	0.000	3.992	0.7770	0.5095	1.0463	0.7558
	F	975	18.13	0.0284	0.0046	0.0018	0.0012	0.0003
	FV	--	18.29	0.1889	0.0449	0.0183	0.0110	0.0021
5	M	1550	0.000	4.368	0.8144	0.6180	0.9713	0.8240
	MV	--	0.000	3.538	0.6976	0.4527	0.9055	0.6743
	F	975	18.31	0.0602	0.0183	0.0067	0.0073	0.0006
	FV	--	16.88	0.1941	0.0471	0.0240	0.0220	0.0037
10	M	1550	0.000	3.716	0.7007	0.5502	0.8122	0.6924
	MV	--	0.000	3.774	0.7565	0.5004	0.9970	0.7111
	F	975	18.29	0.1535	0.0349	0.0177	0.0140	0.0026
	FV	--	17.60	0.2787	0.0752	0.0366	0.0390	0.0035
15	M	1550	0.000	3.874	0.7466	0.5464	0.8488	0.7038
	MV	--	0.000	3.814	0.7434	0.6090	0.7756	0.7141
	F	975	15.90	0.2199	0.0500	0.0299	0.0189	0.0019
	FV	--	16.73	0.3795	0.1069	0.0617	0.0439	0.0052
20	M	1550	0.074	3.794	0.7387	0.6145	0.7994	0.7204
	MV	--	--	--	--	--	--	--
	F	975	18.09	0.2855	0.0805	0.0378	0.0384	0.0061
	FV	--	--	--	--	--	--	--
30	M	1580	0.342	3.338	0.6578	0.5006	0.8396	0.6591
	MV	--	0.496	3.258	0.6429	0.5761	0.7500	0.6119
	F	905	18.00	0.4705	0.1610	0.0775	0.0732	0.0086
	FV	--	16.89	0.5300	0.1996	0.1044	0.0939	0.0128
40	M	1635	0.669	3.178	0.6385	0.5651	0.7207	0.6259
	MV	--	0.609	3.158	0.6018	0.5615	0.7244	0.6002
	F	885	18.36	0.5276	0.2095	0.1037	0.1000	0.0126
	FV	--	17.99	0.5299	0.2345	0.1190	0.1070	0.0150
60	M	1690	0.888	2.821	0.5656	0.5303	0.6860	0.5767
	MV	--	0.829	2.762	0.5527	0.4733	0.6585	0.5670
	F	865	19.03	0.5169	0.2747	0.1434	0.1531	0.0188
	FV	--	16.78	0.4187	0.2760	0.1606	0.1555	0.0192
80	M	1745	0.927	2.544	0.5383	0.5175	0.6512	0.5717
	MV	--	1.008	2.523	0.5223	0.5102	0.6146	0.5129
	F	845	19.11	0.4044	0.3132	0.1783	0.1902	0.0256
	FV	--	16.72	0.4124	0.3175	0.1930	0.1939	0.0256
100	M	1775	1.168	2.226	0.5223	0.5084	0.5872	0.5013
	MV	--	1.124	2.226	0.4911	0.4514	0.5972	0.4923
	F	775	16.85	0.3685	0.3436	0.1917	0.2366	0.0313
	FV	--	17.83	0.3595	0.3409	0.2162	0.2277	0.0337
120	M	1805	1.184	2.068	0.4976	0.4772	0.6037	0.4931
	MV	--	1.272	2.067	0.4747	0.4699	0.5707	0.4745
	F	755	17.34	0.3691	0.3623	0.2180	0.2579	0.0339
	FV	--	16.31	0.3491	0.3606	0.1806	0.2967	0.0352
138	M	2360	2.976	1.721	0.4661	0.4331	0.5305	0.3976
	MV	--	--	--	--	--	--	--
	F	145	19.37	0.3304	0.3821	0.2235	0.2963	0.0384
	FV	--	--	--	--	--	--	--
	AF	1300	0.016	0.0517	0.0288	0.0152	0.0134	0.0026

TABLE XII

Volumes Measured and Concentrations of Substances Determined in Samples of Fluids from Placenta 54.

TIME	FLUID	VOLUME	DEX	GLU	ANT	SDM	ASL	BSP
min		ml	mg/ml	µM/ml	µM/ml	µM/ml	µM/ml	µM/ml
0	M	1000	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	MV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	F	1500	19.86	10.08	0.9512	0.7324	1.1122	0.9690
	FV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	AF	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
2	M	950	0.000	0.0725	0.0122	0.0006	0.0031	0.0009
	MV	--	0.004	0.1251	0.0221	0.0042	0.0000	0.0013
	F	1475	17.71	8.93	0.8707	0.6872	1.0122	0.8500
	FV	--	17.29	6.455	0.6274	0.5921	0.6976	0.5575
5	M	900	0.021	0.1316	0.0244	0.0036	0.0000	0.0020
	MV	--	0.076	0.4146	0.0348	0.0232	0.0031	0.0000
	F	1460	16.80	8.487	0.7826	0.6457	0.8537	0.7577
	FV	--	18.42	7.344	0.6948	0.5870	0.8390	0.5964
10	M	900	0.109	0.2562	0.0411	0.0103	0.0067	0.0038
	MV	--	0.121	0.3155	0.0426	0.0158	0.0073	0.0042
	F	1400	18.63	8.344	0.7885	0.6604	0.8878	0.7155
	FV	--	17.83	7.216	0.6604	0.6338	0.7293	0.4553
15	M	900	0.222	0.4005	0.0548	0.0164	0.0171	0.0068
	MV	--	0.328	0.5316	0.0676	0.0299	0.0213	0.0084
	F	1400	18.80	8.212	0.7761	0.6727	0.8659	0.6287
	FV	--	16.91	7.802	0.7365	0.6605	0.8049	0.5732
20	M	1000	0.490	0.6689	0.0874	0.0396	0.0323	0.0124
	MV	--	0.595	0.8198	0.1048	0.0506	0.0400	0.0236
	F	1400	16.68	7.812	0.7729	0.6776	0.8317	0.6108
	FV	--	16.66	7.513	0.6879	0.6361	0.8000	0.5261
30	M	1000	0.731	1.063	0.1396	0.0726	0.0561	0.0220
	MV	--	0.745	1.102	0.1388	0.0763	0.0579	0.0236
	F	1400	17.62	7.112	0.6906	0.6607	0.7756	0.5178
	FV	--	16.74	6.959	0.6301	0.6167	0.7902	0.4971
40	M	1000	0.915	1.292	0.1630	0.0934	0.848	0.0302
	MV	--	1.104	1.363	0.1784	0.0872	0.0988	0.0323
	F	1350	17.82	7.295	0.6753	0.6583	0.8073	0.5150
	FV	--	17.52	6.797	0.6328	0.6412	0.7220	0.4719
60	M	1150	2.444	2.014	0.3033	0.1746	0.1549	0.0647
	MV	--	2.467	2.119	0.2817	0.1857	0.1707	0.0653
	F	1000	16.64	6.670	0.6116	0.6462	0.7610	0.4430
	FV	--	18.81	6.314	0.5811	0.6193	0.7049	0.4083
80	M	1100	3.000	2.301	0.3027	0.2145	0.2030	0.0755
	MV	--	3.192	2.352	0.3211	0.2114	0.2037	0.0757
	F	850	17.33	6.113	0.5778	0.6364	0.7073	0.3787
	FV	--	19.66	5.730	0.5415	0.6096	0.6659	0.3560
100	M	1250	3.936	2.716	0.3570	0.2671	0.2579	0.0896
	MV	--	4.048	2.768	0.3539	0.2652	0.2403	0.0898
	F	800	17.24	5.270	0.5140	0.6072	0.6195	0.3125
	FV	--	18.52	4.814	0.4807	0.5827	0.5793	0.2823
120	M	1325	4.722	2.817	0.4206	0.3079	0.3148	0.1015
	MV	--	4.633	2.870	0.3960	0.3129	0.2976	0.1018
	F	775	18.28	4.473	0.4780	0.5606	0.6195	0.2387
	FV	--	18.71	4.102	0.4534	0.5656	0.5598	0.2114

TABLE XII (continued)

TIME	FLUID	VOLUME	DEX	GLU	ANT	SDM	ASL	BSP
min		ml	mg/ml	μM/ml	μM/ml	μM/ml	μM/ml	μM/ml
140	M	1400	5.272	2.893	0.4320	0.3632	0.3354	0.1113
	MV	--	5.330	2.787	0.4047	0.3498	0.3341	0.1085
	F	600	18.68	3.838	0.4478	0.5313	0.5317	0.1828
	FV	--	20.09	3.487	0.4234	0.5264	0.5476	0.1729
160	M	1425	5.338	2.734	0.4255	0.3895	0.3459	0.1166
	MV	--	5.994	2.730	0.4013	0.3678	0.3651	0.1164
	F	475	21.89	3.081	0.4145	0.5068	0.4610	0.1315
	FV	--	19.99	2.750	0.4026	0.4848	0.5122	0.1240
180	M	1500	6.604	2.647	0.4526	0.4184	0.3854	0.1210
	MV	--	6.429	2.622	0.3949	0.3907	0.3813	0.1210
	F	400	20.51	2.299	0.4059	0.4676	0.4195	0.1010
	FV	--	23.27	2.045	0.4030	0.4602	0.4744	0.0951
	AF	2300	1.061	0.6852	0.1058	0.0825	0.0811	0.0250

TABLE XIII

Volumes Measured and Concentrations of Substances Determined in Samples of Fluids from Placenta 55.

TIME	FLUID	VOLUME	CONCENTRATIONS					
			DEX	GLU	ANT	SDM	ASL	BSP
min		ml	mg/ml	μM/ml	μM/ml	μM/ml	μM/ml	μM/ml
0	M	1000	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	MV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	F	1500	18.85	9.792	0.9592	0.7029	1.0927	0.9673
	FV	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
	AF	--	0.000	0.0000	0.0000	0.0000	0.0000	0.0000
2	M	1100	0.006	0.0329	0.0061	0.0012	0.0000	0.0007
	MV	--	0.037	0.1711	0.0190	0.0079	0.0000	0.0008
	F	1500	16.11	9.019	0.8742	0.6553	1.0000	0.8683
	FV	--	16.55	6.302	0.6159	0.5406	0.6902	0.5308
5	M	1150	0.138	0.2429	0.0273	0.0153	0.0061	0.0027
	MV	--	0.421	0.5705	0.0568	0.0360	0.0232	0.0100
	F	1350	15.77	8.784	0.8436	0.6553	0.9317	0.8134
	FV	--	15.37	7.548	0.7252	0.5968	0.8049	0.5894
10	M	1300	1.047	0.8368	0.0857	0.0470	0.0451	0.0140
	MV	--	1.616	1.288	0.1232	0.0948	0.0793	0.0243
	F	1100	16.28	8.412	0.8133	0.6407	0.9415	0.7576
	FV	--	16.03	8.097	0.7833	0.5991	0.8805	0.6309
15	M	1600	2.741	1.703	0.1588	0.1180	0.1146	0.0324
	MV	--	3.339	2.153	0.2041	0.1577	0.1549	0.0420
	F	850	17.11	8.459	0.8348	0.6333	0.9244	0.7053
	FV	--	17.41	7.535	0.7647	0.6163	0.8634	0.5188
20	M	1800	4.043	2.314	0.2208	0.2195	0.1579	0.0466
	MV	--	4.898	2.803	0.2646	0.2017	0.2177	0.0576
	F	700	17.20	7.853	0.8049	0.5867	0.9463	0.6360
	FV	--	16.54	7.751	0.7431	0.6286	0.8768	0.5040
25	M	2050	4.912	2.789	0.2735	0.2195	0.2006	0.0560
	MV	--	5.803	3.179	0.3220	0.2503	0.2723	0.0652
	F	600	16.94	8.065	0.7673	0.6433	0.8805	0.5417
	FV	--	1609	7.728	0.7247	0.6336	0.8024	0.4286
30	M	2200	5.445	3.181	0.3295	0.2633	0.2569	0.0666
	MV	--	6.318	3.308	0.3416	0.2702	0.3037	0.0717
	F	130	18.00	7.584	0.7307	0.6409	0.8146	0.3851
	FV	--	17.97	7.294	0.7092	0.6434	0.7732	0.3463
	AF	1900	0.021	0.0526	0.0107	0.0030	0.0006	0.0000

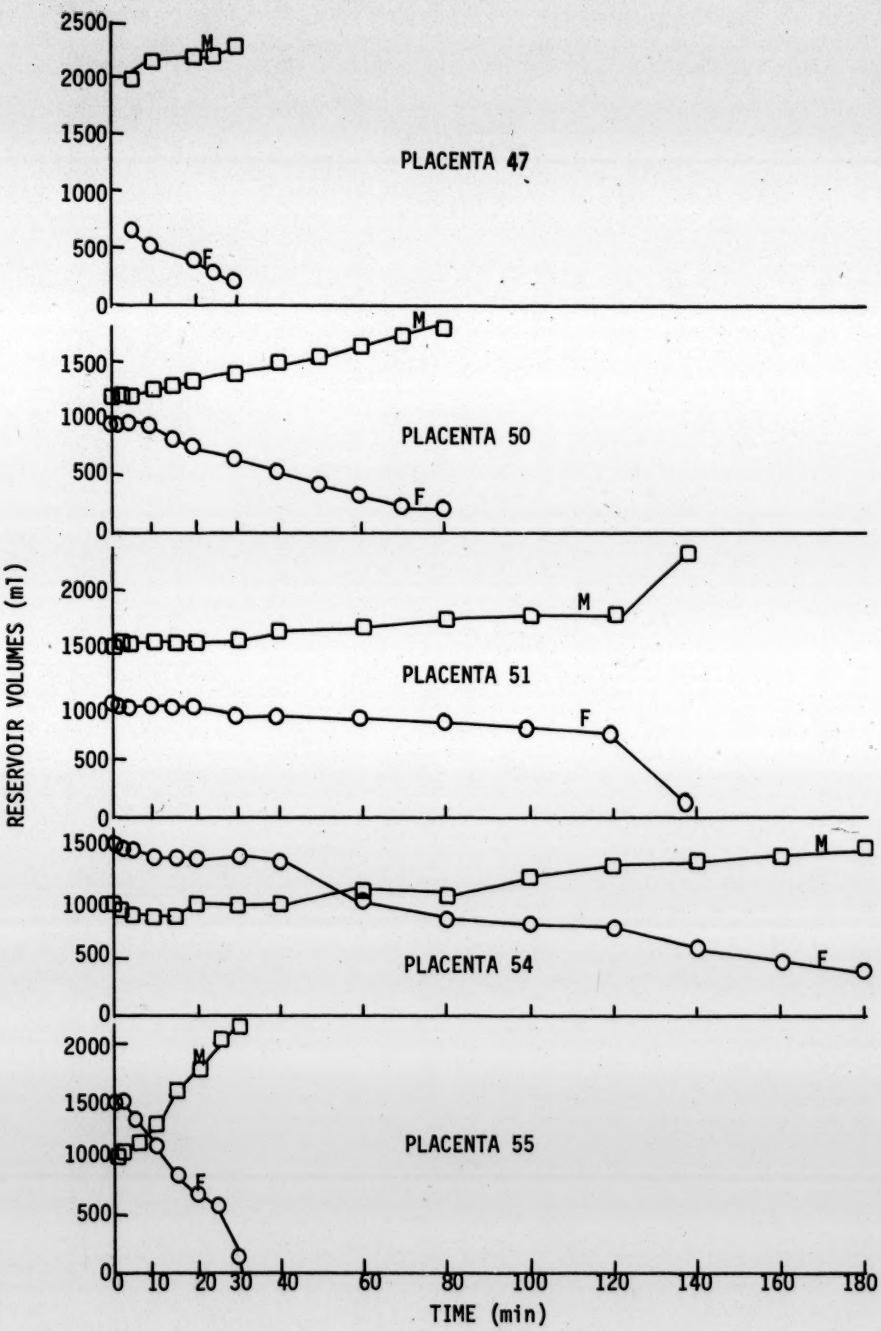


Figure 31. Fetal and maternal reservoir volumes during perfusion of placentas 47, 50, 51, 54 and 55.

concentration, at the times of sampling, in figure 32. The percentages of the total amount calculated for each perfusate and in the amniotic chamber fluid, and the percentages not accounted for at each time point, are plotted in figure 33. DEX was present in the maternal perfusate of placenta 47 at the start of the experiment because rapid fetal to maternal fluid flow took place during the pre-experimental period of perfusion. DEX had been added to the fetal perfusate during this period, consequently, a large amount of DEX had transferred with the fluid to the maternal side. DEX was added at the start of the other experiments rather than during the pre-experimental perfusion periods, so this problem occurred only with placenta 47. Total amounts of DEX were not calculated for placenta 47 since samples were not collected from the maternal or fetal venous circuits.

The disposition of DEX during these experiments was related to the rate of fetal to maternal fluid flow in a manner which was in agreement with the findings of the previous experiments. During those periods of the experiments in which the rate of fetal to maternal fluid flow was less than 2 ml/min, fetal DEX concentrations remained essentially constant after an initial drop due to dilution of the fetal reservoir contents by the recirculating fluid used to prime the apparatus, and to the initial loss of about 10% of the smaller, permeable DEX fractions.

During periods in which the fetal to maternal fluid flow rate exceeded 2 ml/min, DEX concentrations in the fetal reservoir increased with a continuous loss of DEX from the fetal

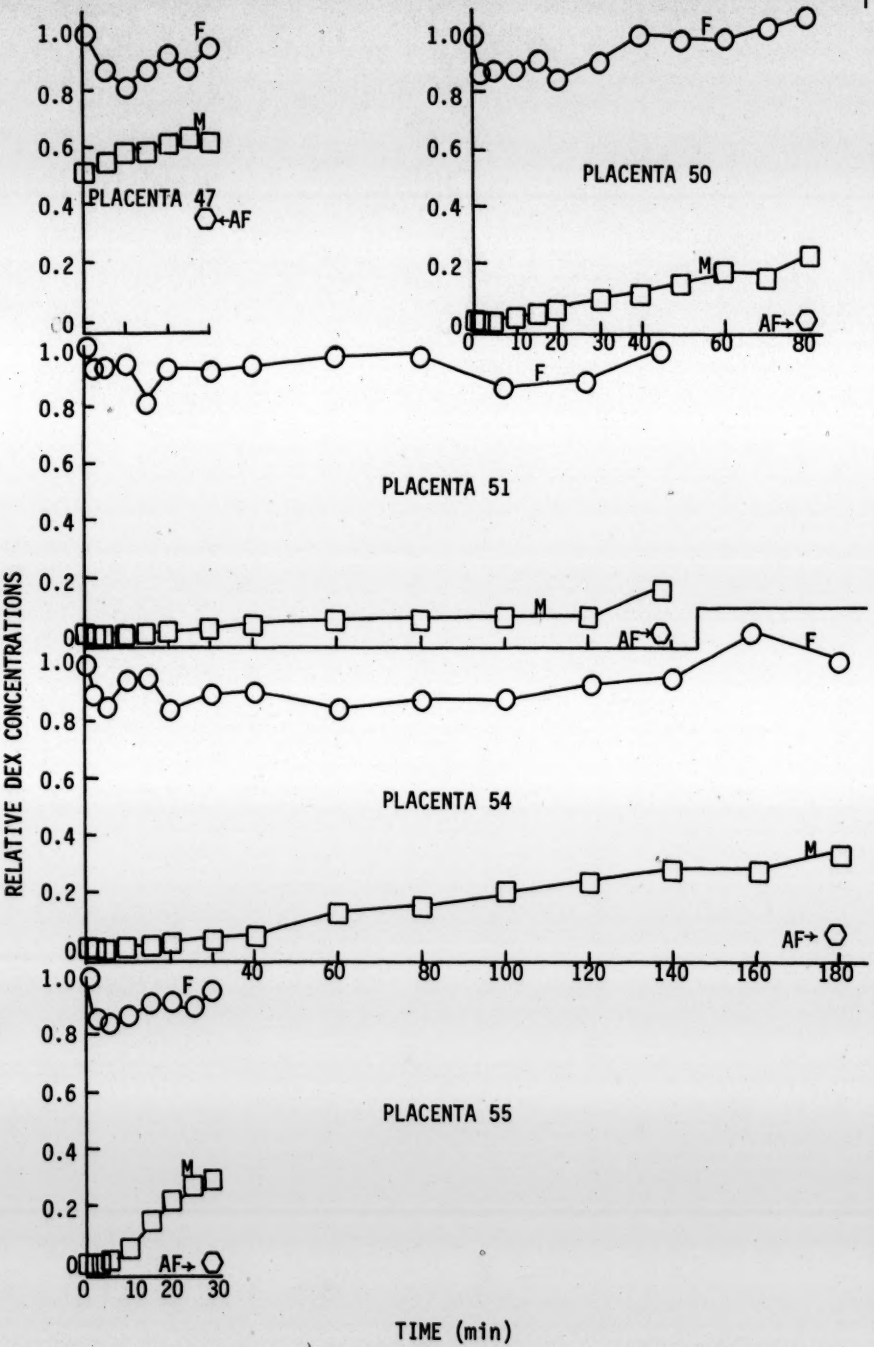


Figure 32. Concentrations of DEX relative to the initial fetal perfusate concentration in fetal reservoir perfusate (F), maternal reservoir perfusate (M) and amniotic chamber fluid (AF) during perfusion of placentas 47, 50, 51, 54 and 55.

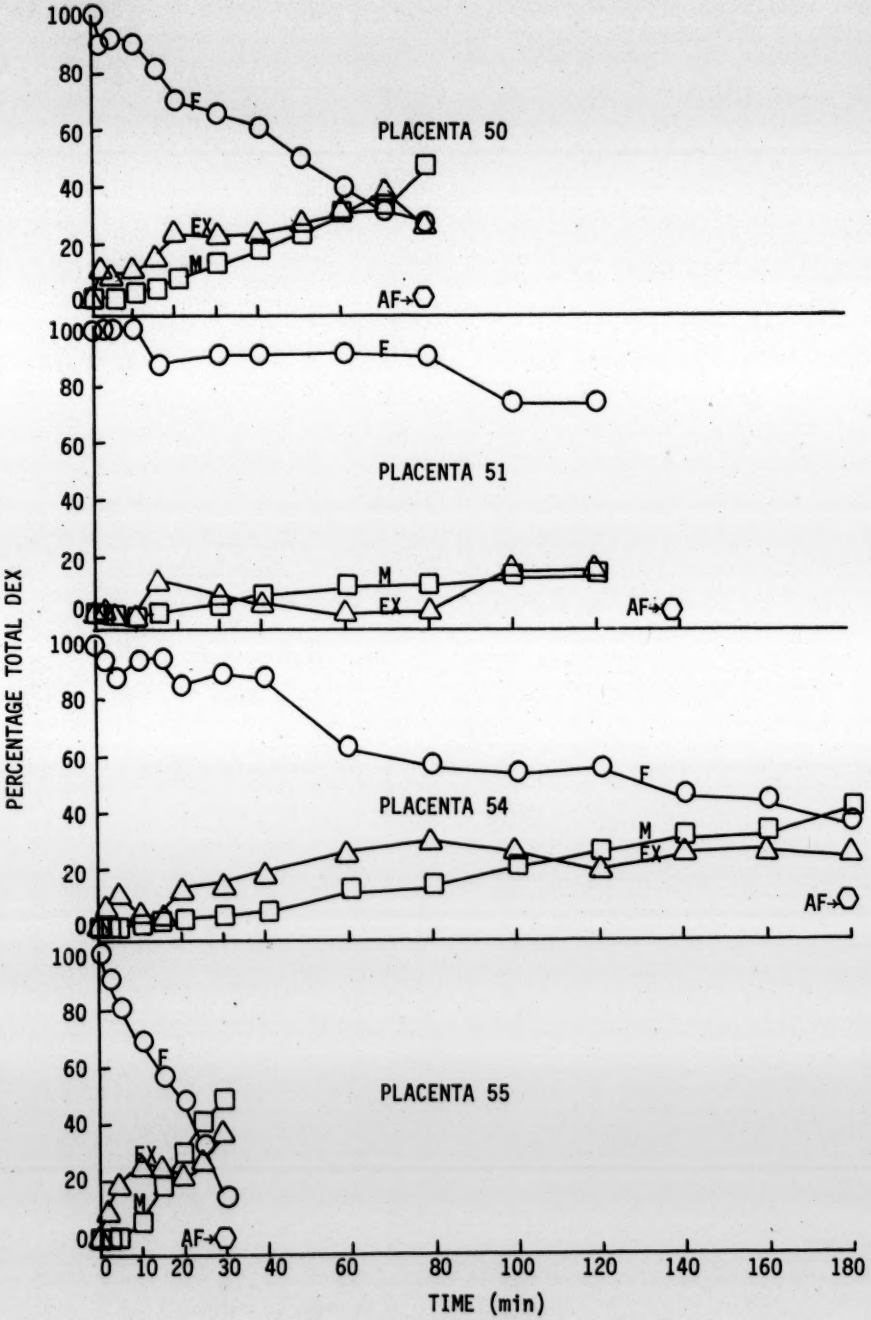


Figure 33. Percentage of total DEX accounted for in the fetal circuit (F), maternal circuit (M), amniotic chamber fluid (AF), and amounts not accounted for in perfusates (EX) during perfusion of placentas 50, 51, 54 and 55.

circuit. These observations of rapid fetal to maternal fluid flow, together with continued loss of greater than 90% of the DEX from the fetal circuit, provide evidence for the conclusion that significant loss of placental viability occurred during the perfusion experiments with the exception of the initial 10 minutes for placenta 50, 80 minutes for placenta 51, and 40 minutes for placenta 54.

In spite of placental viability loss, the placentas showed metabolic activity as evidenced by the disappearance of GLU. Total amounts of GLU accounted for during the perfusions of placentas 50, 51, 54, and 55 are shown in figure 34. There is an immediate rapid decline in the amount of GLU, followed by a second, slower rate of decline evident after 15-20 minutes. This second declining phase was assumed to take place at a constant rate and the data points after the first 20 minutes were fit to an equation for a straight line by linear regression analysis. The regression coefficient (slope), correlation coefficient, and the disappearance rate, calculated from the slope, are listed in table XIV.

The calculated disappearance rates during these experiments are at the lower limit of the range of values reported for the placenta by other investigators as listed in table VII. As previously discussed, the disappearance rate is only a qualitative indicator of metabolic activity of the placental tissues and is lower than the actual glucose utilization rates, which can only be measured during steady-state.

There does not appear to be significant changes in the disappearance rates of GLU from the system with changes in the

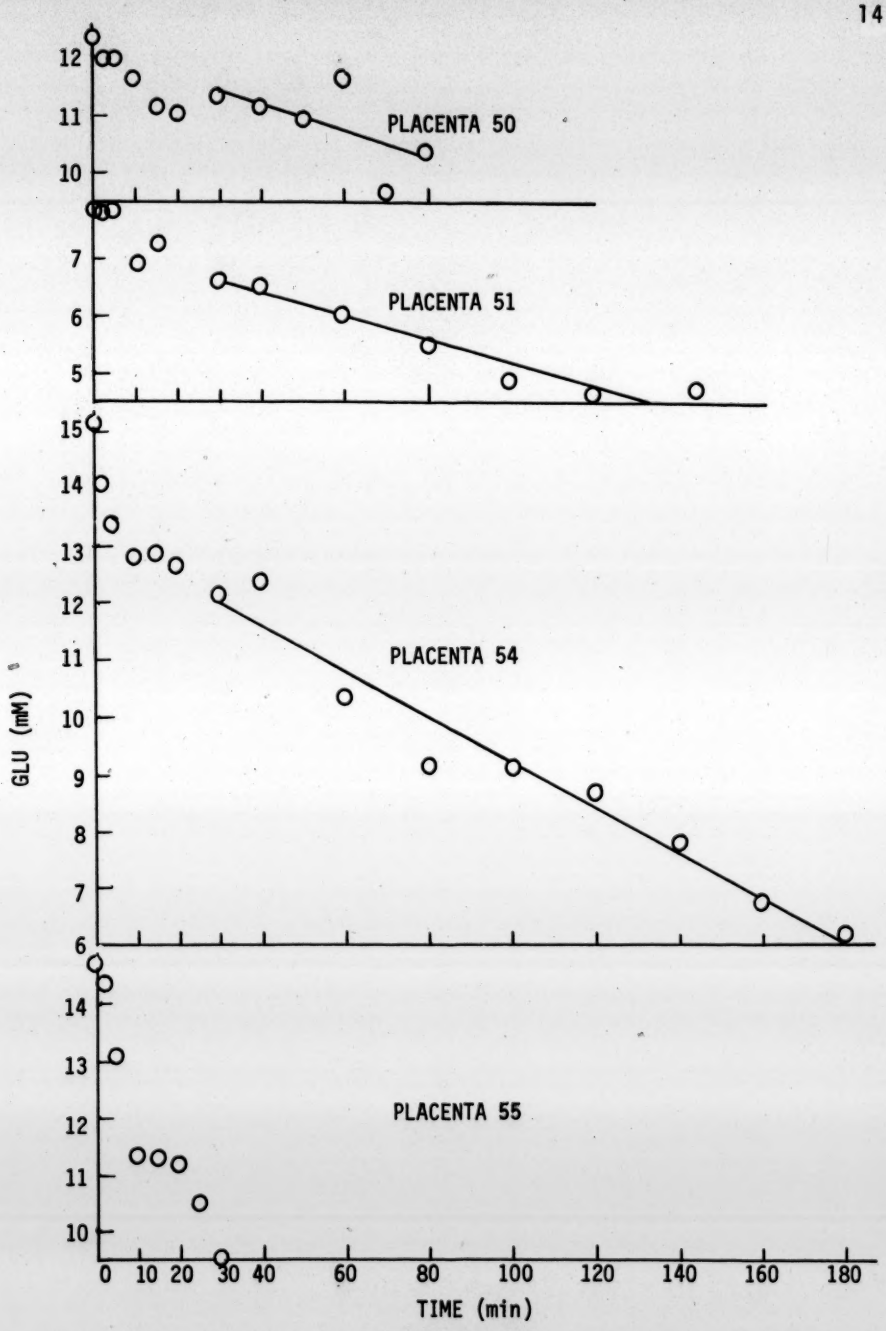


Figure 34. Total amounts of GLU accounted for in perfusates during perfusion of placentas 50, 51, 54 and 55. The straight lines shown are the calculated linear regression lines using data after the first 20 minutes only.

fetal to maternal flow rates.

TABLE XIV

Linear Regression Analysis and Disappearance Rates of GLU During Perfusion of Placentas 50, 51, and 54

Placenta	Regression Coefficient mM/min	Correlation Coefficient	Disappearance Rate uM/g/hr
50	-0.0247	-0.634	2.18
51	-0.0204	-0.976	2.09
54	-0.0397	-0.977	2.65

Disposition of Substances Added to Perfusates

Concentrations of each of the substances added to perfusates determined from samples collected during the experiments are listed in tables IX-XIII. Concentrations of the drugs and of GLU, expressed as ratios of the zero time concentrations determined in the reservoir to which these substances were added (relative concentrations), are plotted at the times the samples were collected in figures 35-40.

Initial disappearance from maternal or fetal reservoir.--The initial disappearance rate of any of the substances from the perfusate to which they were first added is a function of the clearance of the substance from the perfusate into the placenta. Therefore, comparison of the relative concentrations of the test substances in the perfusate to which the substances were added, at times in which transfer back into the perfusate from the placenta is negligible, is a comparative measure of the

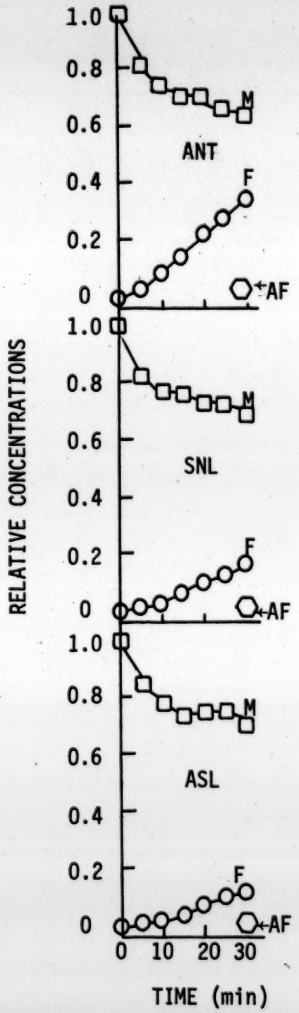


Figure 35. Concentrations of ANT, SNL and ASL, relative to the initial maternal perfusate concentrations, in maternal perfusate (M), fetal perfusate (F), and amniotic chamber fluid (AF) during perfusion of placenta 47.

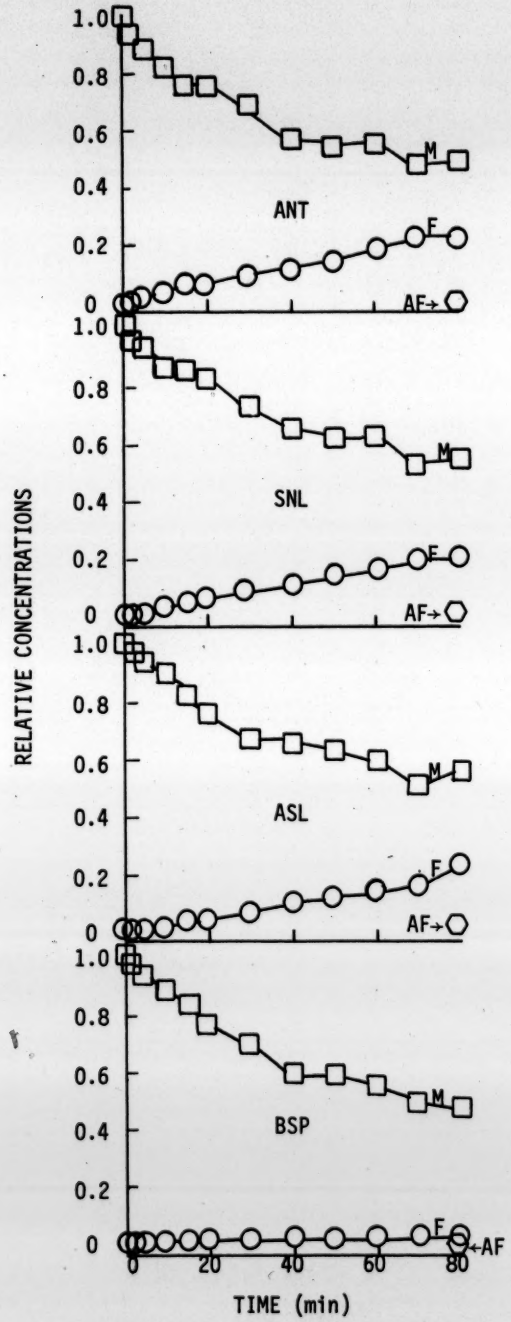


Figure 36. Concentrations of ANT, SNL, ASL and BSP, relative to the initial maternal perfusate concentrations, in maternal perfusate (M), fetal perfusate (F), and amniotic chamber fluid (AF) during perfusion of placenta 50.

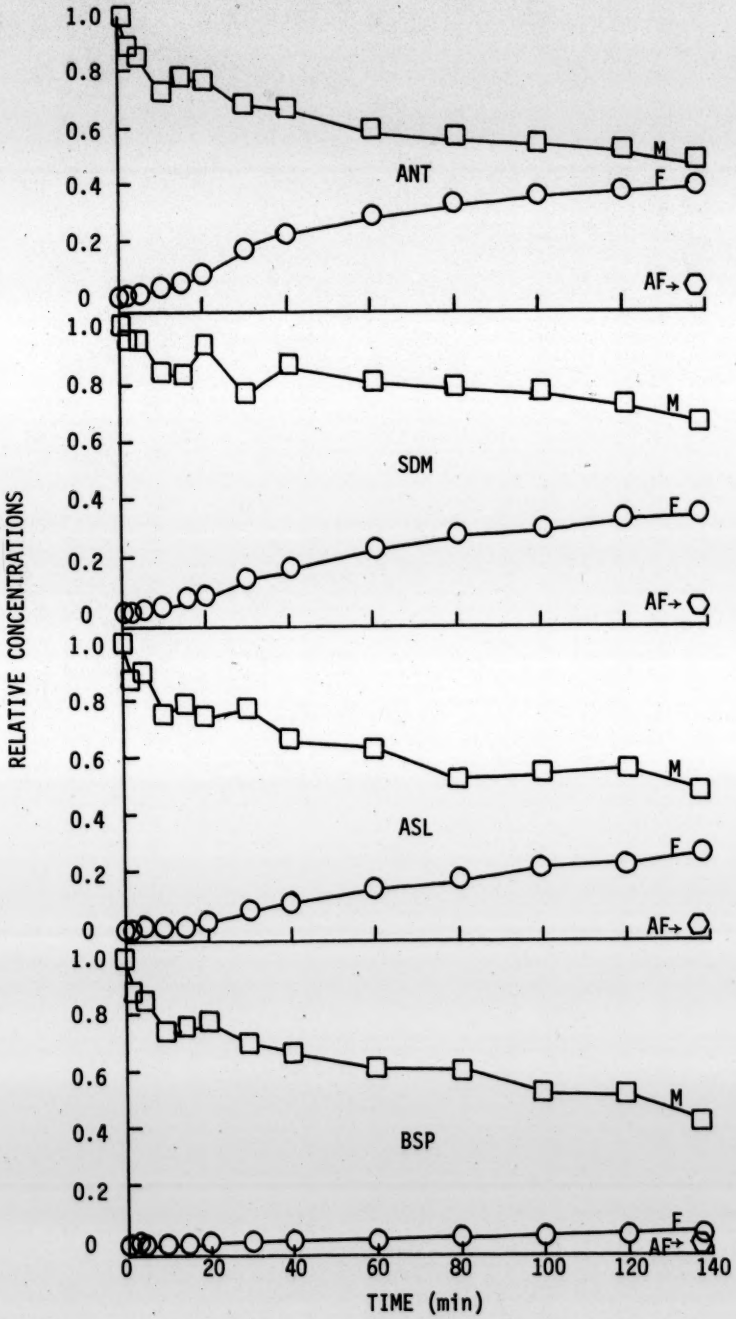


Figure 37. Concentrations of ANT, SDM, ASL and BSP, relative to the initial maternal perfusate concentrations, in maternal perfusate (M), fetal perfusate (F), and amniotic chamber fluid (AF) during perfusion of placenta 51.

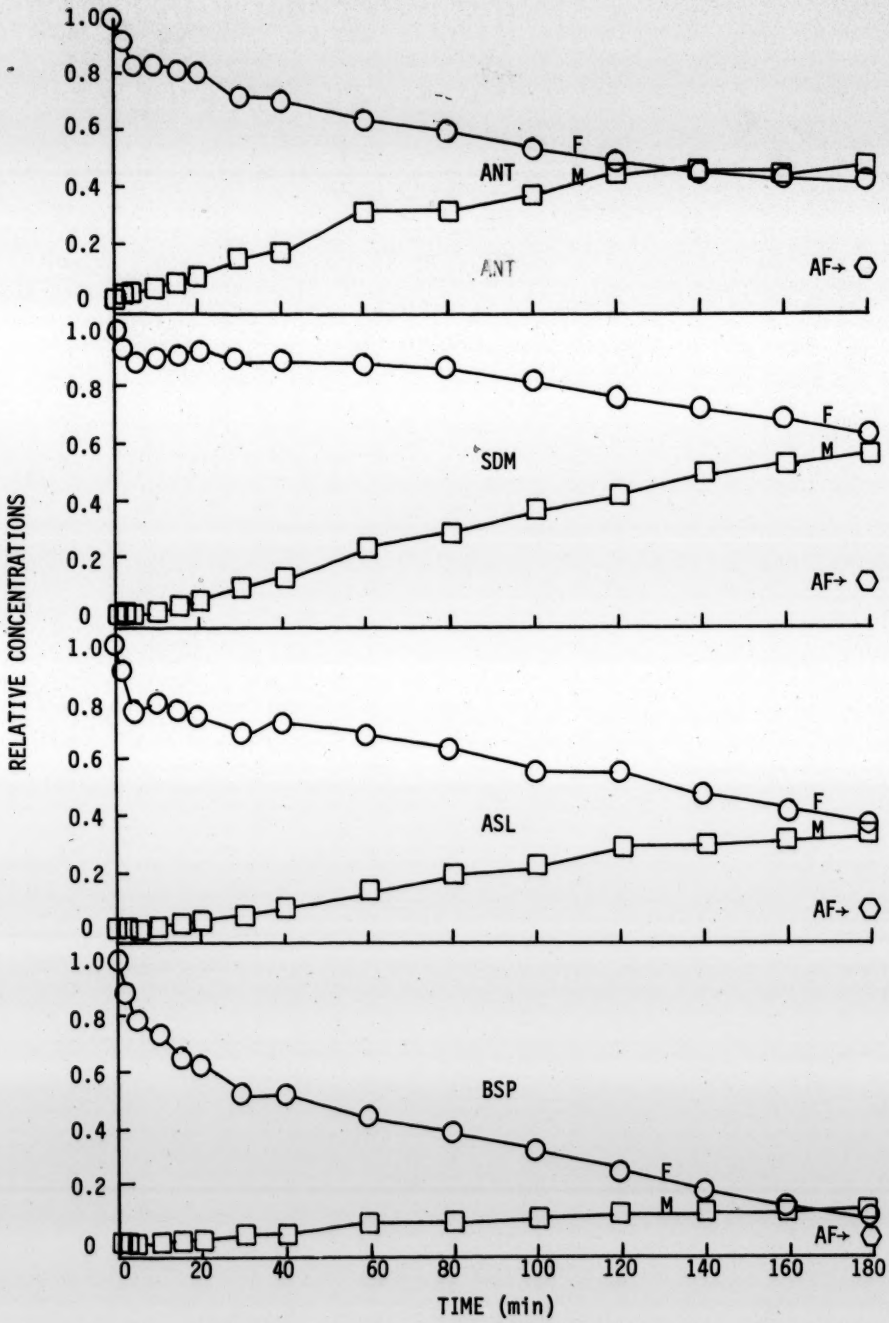


Figure 38. Concentrations of ANT, SDM, ASL and BSP, relative to the initial fetal perfusate concentrations, in fetal perfusate (F), maternal perfusate (M), and amniotic chamber fluid (AF) during perfusion of placenta 54.

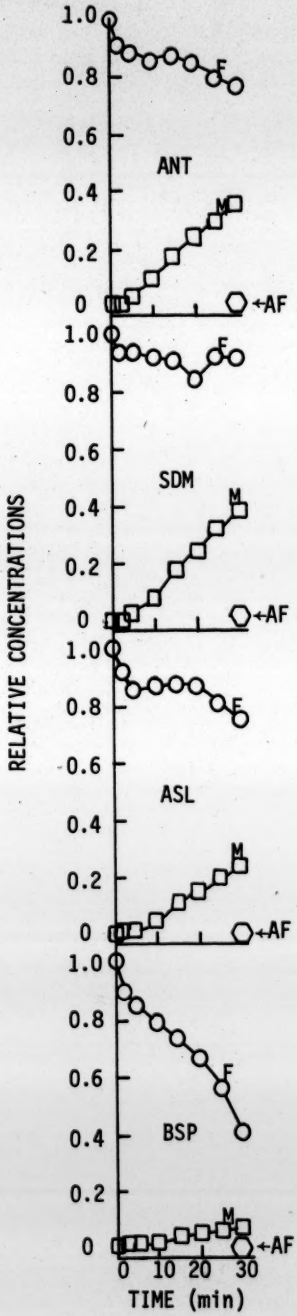


Figure 39. Concentrations of ANT, SDM, ASL and BSP, relative to the initial fetal perfusate concentrations, in fetal perfusate (F), maternal perfusate (M), amniotic chamber fluid (AF) during perfusion of placenta 55.

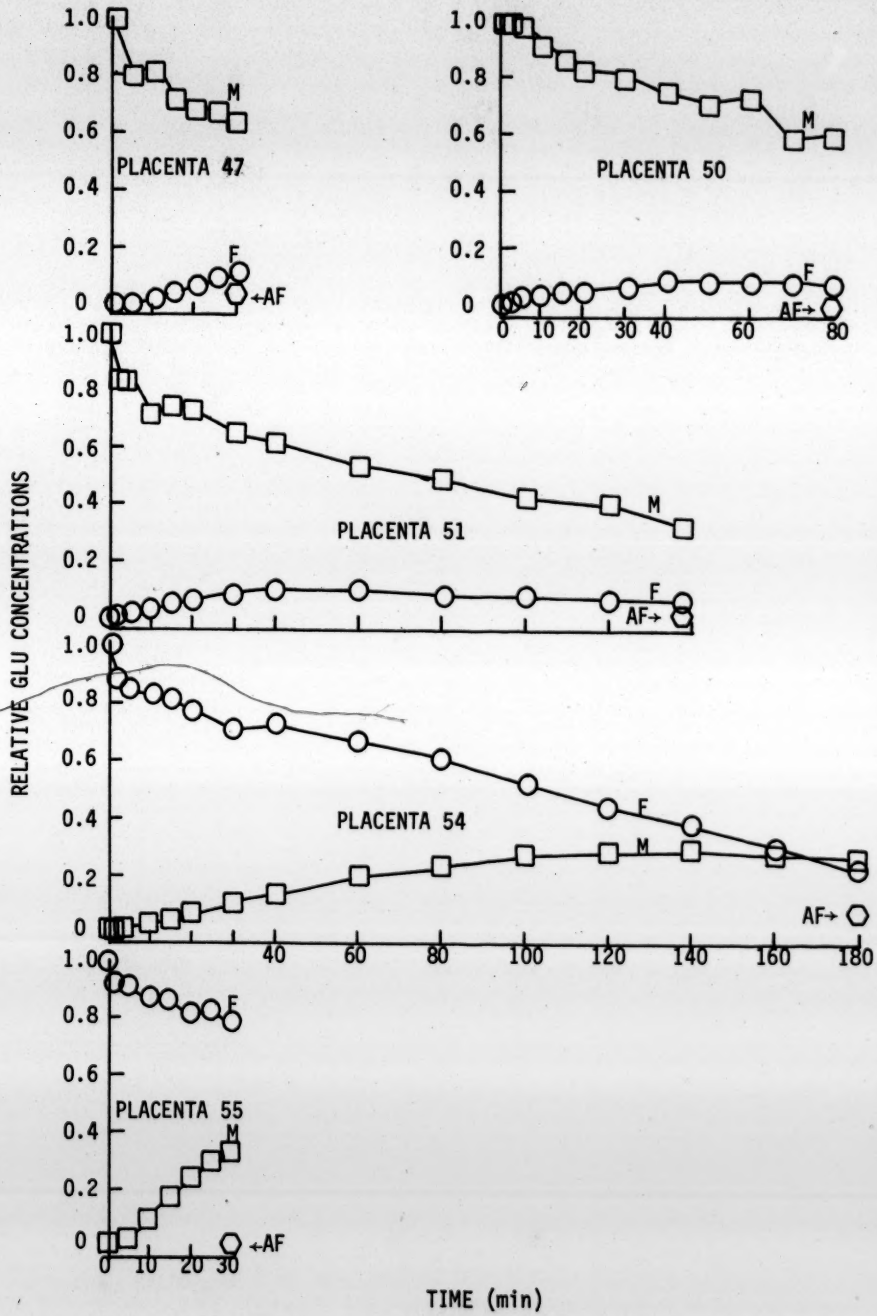


Figure 40. Concentrations of GLU, relative to the initial maternal or fetal perfusate concentrations, in maternal perfusates (M), fetal perfusates (F), and amniotic chamber fluids (AF) during perfusion of placentas 47, 50, 51, 54 and 55.

clearances of the substances; that is, the greater the clearance of the substance from the perfusate into the placenta, the lower will be the relative concentration of that substance in the perfusate.

Comparison of the relative concentrations of the drugs and GLU at 2, 5, and 10 minutes after addition to either the maternal or fetal reservoirs, may be made by examination of figures 35-40, or by reference to table XV in which the data are listed. These data show that for the experiments in which the test substances were initially added to the maternal reservoir, relative concentrations of the various substances are not significantly different over the first ten minutes, their values falling within the range of error due to sampling and assay. The one exception to this statement appears to be with SDM in the experiment with placenta 51. The relative concentrations of SDM in the maternal perfusate during the experiment rose and fell periodically. This erratic behavior was due to the low solubility and/or slow dissolution rate of SDM which did not completely dissolve in the perfusate at the start of the experiment. In subsequent experiments which employed SDM as a test substance, perfusates were filtered before use to remove any undissolved SDM. The fact that no significant differences were observed in the relative concentrations during the first 10 minutes of the various test substances after their addition to the maternal reservoir, suggests that the clearances of these substances from maternal perfusate into the placenta are limited by the rate of maternal perfusate flow, rather than by diffusion of the substances from the perfusate into the placenta. If

TABLE XV

Disappearance of Test Substances from Reservoir to which Initially Added

TIME	SUBSTANCE	RELATIVE CONCENTRATION IN RESERVOIR OF PLACENTA							
		MATERNAL			FETAL				
		47	50	51	47	50	51	54	55
min 2	ANT	--	.949	.883	--	--	--	.915	.911
	SNL	--	.964	--	--	--	--	--	--
	SDM	--	--	.948	--	--	--	.938	.932
	ASL	--	.981	.872	--	--	--	.910	.915
	BSP	--	.999	.882	--	--	--	.877	.898
	GLU	--	.995	.830	--	--	--	.886	.921
	DEX	--	--	--	--	.868	.928	.892	.855
5	ANT	.813	.879	.851	--	--	--	.823	.879
	SNL	.824	.921	--	--	--	--	--	--
	SDM	--	--	.945	--	--	--	.882	.932
	ASL	.844	.928	.904	--	--	--	.768	.853
	BSP	--	.936	.849	--	--	--	.782	.841
	GLU	.805	.981	.837	--	--	--	.842	.897
	DEX	--	--	--	.875	.876	.937	.856	.837
10	ANT	.756	.824	.733	--	--	--	.829	.848
	SNL	.774	.874	--	--	--	--	--	--
	SDM	--	--	.841	--	--	--	.902	.912
	ASL	.782	.879	.756	--	--	--	.798	.862
	BSP	--	.884	.741	--	--	--	.738	.783
	GLU	.825	.914	.713	--	--	--	.828	.859
	DEX	--	--	--	.818	.879	.936	.938	.864

these clearances were diffusion limited, one would see differences in the relative concentrations based on the physical properties of the substances.

Differences in relative fetal perfusate concentrations of substances which were simultaneously added to the fetal reservoir at the initial time of experiment are also not significant since concentrations fell within the range of assay error, although definite trends are evident. Relative concentrations of SDM are consistently higher, and relative concentrations of BSP and ASL are consistently lower than those observed for ANT or GLU, (figures 38-40, table XV). This trend might indicate that the clearance from the fetal perfusate into the placenta is highest for BSP and ASL, and lowest for SDM. A more likely explanation is that transfer back from the placenta to the fetal perfusate is not negligible for SDM, ANT, and GLU, but is insignificant for BSP and ASL, and that there are no differences in the clearances from fetal perfusates into the placenta for the various test substances. This second possibility seems more likely since both ANT and GLU are known to transfer rapidly across a variety of biological membranes.

There appeared to be no qualitative differences in the results observed in experiments in which fetal to maternal fluid flow was rapid, compared to results observed when fetal to maternal fluid flow was negligible.

Initial appearance in fetal or maternal reservoir.--In each experiment, the test substances were detected in the reservoir opposite the reservoir to which they were initially added within two minutes. Differences in relative concentrations of

the test substances during the first ten minutes, as listed in table XVI, reflect differences in rates of appearance in the perfusate from the placenta. In decreasing order, the rank order of appearance in the fetal reservoir was GLU, ANT, SNL or SDM, ASL, and BSP for the experiments in which these substances were initially added to the maternal reservoir. Similar rank order of appearance in the maternal reservoir when the test substances were added initially to the fetal reservoir was observed. Initial appearance rates for DEX in the maternal reservoir varied with each experiment depending on the rates of fetal to maternal fluid flow rates. The more rapid the fetal to maternal fluid flow rate, the more rapid was the appearance rate of DEX in the maternal perfusate. The rank order of appearance rates of the other substances did not change with the rate of fetal to maternal fluid flow.

The observation that rates of appearance of the test substances in the perfusate opposite to which they were initially added were significantly different while no significant differences were found in their rates of disappearance from the reservoir to which they were initially added suggest a limitation to diffusion within the placenta.

Placental uptake.--Differences in the amounts of the substances studied which cannot be accounted for in the perfusates during the placental perfusion experiments are also apparent. These amounts of ANT, SNL or SDM, ASL, BSP, and GLU, expressed as percentages of total amounts in the system at each time point, are plotted in figure 41 for placentas 50, 51, 54, and 55. The values plotted for placenta 51, which are negative

TABLE XVI

Appearance of Test Substances in Reservoir Opposite to which initially Added

TIME	SUBSTANCE	RELATIVE CONCENTRATION IN RESERVOIR OF PLACENTA							
		FETAL			MATERNAL				
		47	50	51	47	50	51	54	55
min									
2	ANT	--	.00072	.0048	--	--	--	.0128	.0064
	SNL	--	.00066	--	--	--	--	--	--
	SDM	--	--	.0028	--	--	--	.0008	.0017
	ASL	--	.0000	.0011	--	--	--	.0028	.0000
	BSP	--	.00057	.0003	--	--	--	.0009	.0008
	GLU	--	.0178	.0054	--	--	--	.0072	.0034
	DEX	--	--	--	--	.0000	.0000	.0000	.0037
5	ANT	.035	.0123	.0191	--	--	--	.0257	.0285
	SNL	.016	.0086	--	--	--	--	--	--
	SDM	--	--	.0102	--	--	--	.0049	.0218
	ASL	.008	.0027	.0068	--	--	--	.0000	.0056
	BSP	--	.0011	.0006	--	--	--	.0021	.0100
	GLU	.000	.0234	.0176	--	--	--	.0131	.0248
	DEX	--	--	--	.560	.0000	.0000	.0011	.0421
10	ANT	.090	.0279	.0365	--	--	--	.0432	.0894
	SNL	.036	.0243	--	--	--	--	--	--
	SDM	--	--	.0271	--	--	--	.0141	.0669
	ASL	.025	.0130	.0130	--	--	--	.0060	.0413
	BSP	--	.0028	.0130	--	--	--	.0039	.0243
	GLU	.024	.0345	.0292	--	--	--	.0254	.0855
	DEX	--	--	--	.595	.0130	.0000	.0055	.1616

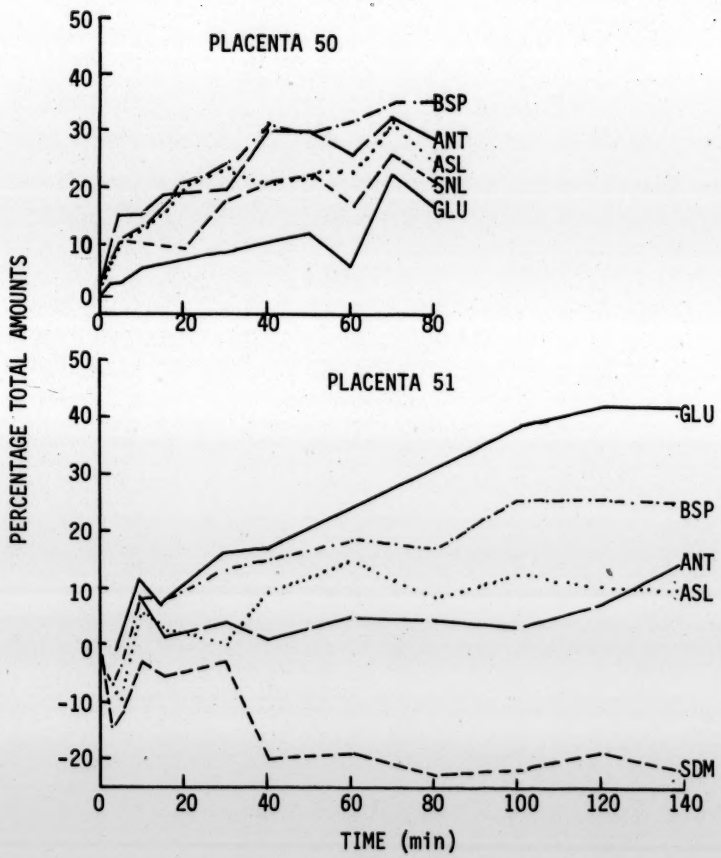


Figure 41. Percentages of total ANT, SNL, SDM, ASL, BSP and GLU not accounted for in maternal and fetal perfusates during perfusion of placentas 50, 51, 54 and 55.

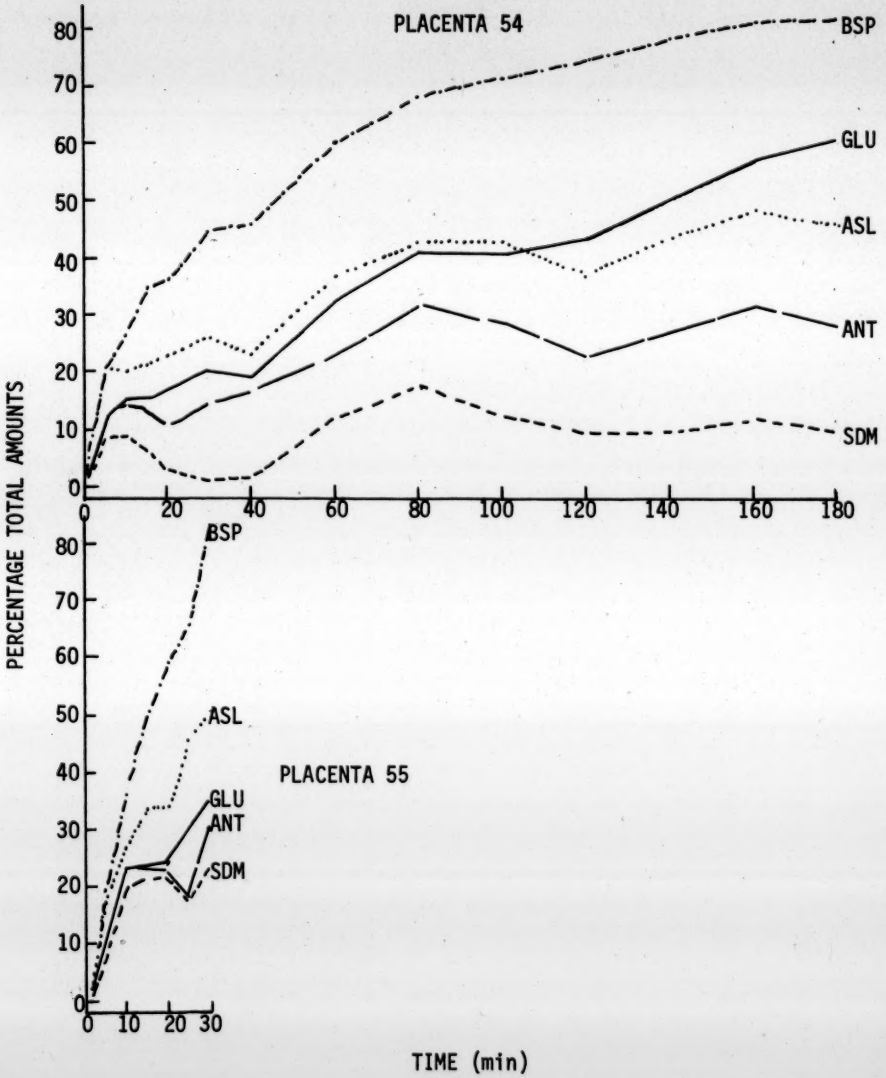


Figure 41. (continued)

in some cases, are no doubt lower than they should be by some constant factor, apparently due to a miscalibration of the reservoir volume measuring device.

Transfer into the amniotic chamber fluid cannot account for the amounts of test substances which are unaccounted for in perfusates. With the exception of placenta 54, amounts of substances in amniotic fluid represented only a minor portion of the unaccounted for substances, table XIX.

The results of perfusion of the maternal and fetal circuits of the apparatus without a placenta are listed in table XVII. Statistical analysis of the data (table XVIII), show that the initial drop in concentrations of the substances are not different than the drop expected by dilution of the reservoir contents by the volume of fluid used to prime the apparatus, and that no trend toward decline in concentration of any of the substances was evident over the three hour period of perfusion. These results rule out the possibility of uptake or destruction of the test compounds by the apparatus, or their instability in the perfusate during the experimental periods.

It is unlikely that placental metabolism of the test substances, other than GLU, occurred to any significant extent during these experiments. Human placental tissue homogenates were not shown to metabolize BSP and SNL in vitro (57,168). Oxidative metabolic reactions to which ANT is susceptible in vivo (149) proceed only slowly if at all in the placenta (168). Acetylation of compounds similar to SDM by placental homogenates has not been demonstrated (57,168). Hydrolysis of N-acetylated compounds, such as ASL, by placental tissue, has not been

TABLE XVII

Concentrations of Substances Determined in Samples from Blank Perfusions

TIME	CONCENTRATION											
	FETAL BLANK PERFUSION						MATERNAL BLANK PERFUSION					
	ANT	SDM	ASL	BSP	GLU	DEX	ANT	SDM	ASL	BSP	GLU	DEX
min	µM/ml	µM/ml	µM/ml	µM/ml	µM/ml	mg/ml	µM/ml	µM/ml	µM/ml	µM/ml	µM/ml	mg/ml
0	0.964	0.775	1.178	0.988	10.37	1.91	0.980	0.370	1.078	0.961	10.45	0.200
2	0.884	0.650	1.102	0.880	9.23	1.65	0.764	0.292	0.873	0.732	7.13	0.171
5	0.895	0.687	1.002	0.886	9.15	1.69	0.733	0.280	0.817	0.726	8.16	0.146
10	0.862	0.665	1.068	0.866	9.12	1.62	0.764	0.290	0.822	0.728	0.835	0.139
15	0.901	0.704	1.102	0.920	9.88	1.71	0.748	0.283	0.820	0.712	8.29	0.129
60	0.889	0.663	1.041	0.897	9.75	1.74	0.772	0.310	0.861	0.752	8.90	0.153
120	0.843	0.685	1.132	0.886	9.62	1.63	0.776	0.295	0.871	0.736	8.48	0.148
180	0.886	0.665	1.039	0.880	9.57	1.72	0.748	0.278	0.795	0.706	8.24	0.141
*MEAN	0.880	0.674	1.069	0.885	9.47	1.68	0.758	0.290	0.837	0.727	8.22	0.147
± SD	0.020	0.019	0.045	0.019	0.31	0.05	0.015	0.011	0.033	0.015	0.54	0.013

*exclusive of zero time data.

TABLE XVIII

Statistical Analysis of Blank Perfusion Data

Question I: Are the concentrations measured in perfusates for the test substances, relative to the initial perfusate concentrations (relative concentrations, C*) different than concentrations expected after dilution by the priming fluid? Answer: The difference is not significant if (DIL-MEAN)/SE,(t) <2.45 (P < 0.05) (189).

FETAL BLANK PERFUSION (DIL = 0.904)					MATERNAL BLANK PERFUSION (DIL = 0.767)				
TEST SUBSTANCE	RELATIVE CONCENTRATIONS		t	SIGNIFICANCE	TEST SUBSTANCE	RELATIVE CONCENTRATIONS		t	SIGNIFICANCE
	MEAN	SE				MEAN	SE		
ANT	0.912	0.008	+1.013	n.s.	ANT	0.774	0.006	+1.167	n.s.
SDM	0.870	0.009	-3.75	sig.(P<0.01)	SDM	0.782	0.011	+1.364	n.s.
ASL	0.908	0.014	+0.278	n.s.	ASL	0.776	0.011	+0.818	n.s.
BSP	0.896	0.007	-0.833	n.s.	BSP	0.757	0.006	-1.667	n.s.
GLU	0.914	0.011	+0.909	n.s.	GLU	0.787	0.022	+0.930	n.s.
DEX	0.882	0.009	-2.53	sig.(P<0.05)	DEX	0.734	0.023	-1.45	n.s.

Question II: Are the variations in concentrations measured between 2 and 180 minutes due to random variation of sampling and assay, or is their a trend toward decreasing concentrations with time, indicating degredation or binding; i.e. is there significant correlation between concentration with time and is the linear regression coefficient significantly different than zero? Answer: There is no significant correlation if the correlation coefficient (r) calculated by linear regression analysis < 0.669 in absolute value; and the calculated regression coefficient is not significantly different than zero if the value of F (1,5 DF) < 6.61 (189).

FETAL BLANK PERFUSION						MATERNAL BLANK PERFUSION					
TEST SUBSTANCE	REGRESSION COEFFICIENT	CORRELATION COEFFICIENT	F	SIGNIFICANCE		TEST SUBSTANCE	REGRESSION COEFFICIENT	CORRELATION COEFFICIENT	F	SIGNIFICANCE	
				REGRESS.	CORREL.					REGRESS.	CORREL.
	μM/ml/min						μM/ml/min				
ANT	-0.00009	-0.31	0.521	n.s.	n.s.	ANT	+0.00004	+0.17	0.140	n.s.	n.s.
SDM	-0.00002	-0.08	0.035	n.s.	n.s.	SDM	-0.00001	-0.09	0.039	n.s.	n.s.
ASL	+0.00002	+0.03	0.004	n.s.	n.s.	ASL	-0.00009	-0.19	0.193	n.s.	n.s.
BSP	-0.00007	-0.27	0.397	n.s.	n.s.	BSP	-0.00006	-0.25	0.330	n.s.	n.s.
GLU	+0.0018	+0.41	0.990	n.s.	n.s.	GLU	+0.0026	+0.33	0.629	n.s.	n.s.
DEX	+0.00016	+0.24	0.300	n.s.	n.s.	DEX	-0.00028	-0.15	0.108	n.s.	n.s.

studied. Acetylsalicylic acid, an O-acetylated compound, was found to be slowly deacetylated in the presence of placental homogenates (57,168).

Further evidence against significant placental metabolism of the test substances other than GLU may be seen by comparison of the disposition patterns of the compounds. The data in figure 41 shows that the amounts of the drugs not accounted for in the perfusates tend to reach a plateau. On the other hand, the amount of GLU which is not accounted for tends to increase during the experiments. Also, the time course of GLU concentration in each of the experiments (figure 40) shows a maximum concentration reached in the perfusate opposite to which GLU was initially added, followed by a decline in concentration with time. Thus, in the case of GLU, there is a net loss of the compound from both perfusates as a consequence of placental tissue metabolism. The concentrations of the drugs in reservoirs opposite to which they were initially added, continue to rise throughout the experiments (figures 35-39), indicating that if metabolism does occur, it takes place at rates much smaller than the rates at which GLU is metabolized.

It must be concluded, therefore, that all the substances studied distribute to a significant extent within the extracellular and intracellular spaces of the placenta.

Of the substances studied, BSP is taken up by the placenta to the greatest extent, and probably is bound to tissue proteins, as was discussed in the previous section. The uptake of BSP by the placenta is more rapid when BSP was initially added to the fetal perfusate, figure 41. This may indicate a

site of BSP binding in the placenta in one of the tissue layers close to the fetal circuit, e.g., the connective tissue layer. However, no tissue distribution studies were made to confirm this.

ANT is known to distribute throughout intracellular and extracellular water of all body organs and is used experimentally to measure body water volume (149). Since 85% of the wet placental weight is water (153), the volume of distribution for ANT in the placentas studied should be significant since weights of placentas used in this study range from 585 to 940 g.

Uptake of SNL and SDM were less than that of ANT, while uptake of ASL was greater. The finding with ASL was not expected because distribution of ASL in various tissues of the cat was found to be less than distribution of SNL in the same tissues (186). It is possible that tissue binding may occur in the placenta for ASL as for BSP, but this has not been reported for ASL in any biological tissues.

Appearance of test substances in amniotic chamber fluid.--The percentage of each of the test substances found in the amniotic chamber fluid at the conclusions of the experiments are listed in table XIX.

It is of interest to speculate as to how the substances gain entry to the amniotic chamber fluid of this ex vivo preparation. In vivo, maternally administered drugs often gain access to the amniotic fluid, the principal route of transfer from the mother being via the fetus, which is absent in this preparation.

TABLE XIX

Percentages of Test Substances Found in Amniotic Chamber Fluid at Conclusions of Experiments

Placenta	Duration of Experiment	Percentage at Conclusion of Experiment						
		ANT	SNL	SDM	ASL	BSP	GLU	DEX
	min							
50	80	0.40	1.07	---	0.39	0.00	0.30	0.02
51	138	2.71	---	2.10	1.11	0.24	0.89	1.13
54	180	17.60	---	19.95	11.50	4.02	10.72	8.50
55	30	1.43	---	0.55	0.07	0.01	0.69	0.14

One possibility is for leakage of fetal perfusate from the cannulas which lead into the umbilical vessels to occur. This type of leakage would cause an increase in the volume of fluid in the amniotic chamber with a noticeable rise in the fluid level. A rise in fluid level was not observed in any of the experiments, thus ruling out this possibility.

Another possibility which may be ruled out is direct leakage of fluid between the placental and amniotic fluid chambers, caused by imperfections in the fetal membranes or rubber sheet used to form a seal between the two chambers, or by their improper placement. If this were the case, at the conclusion of the experiment, amniotic chamber fluid would drain through the venous openings of the placental chamber when the maternal pump was turned off. This type of drainage was not observed.

Transfer of water and various organic and inorganic

substances has been shown to take place across the umbilical cord between the amniotic fluid and the umbilical vein (187). Transfer by this route in these experiments is unlikely, however, since the plastic cannulas inserted in the umbilical vein were secured at the placental insertion of the umbilical cord.

Two remaining possibilities are: 1) transfer from the placental tissue across the chorionic plate and amnion into the amniotic chamber fluid; and 2) transfer from the maternal perfusate contained in the placental chamber across the fetal membranes into the amniotic chamber fluid. Studies of transfer from the placental tissues into the amniotic fluid have not been reported in the literature, but this mechanism can not be ruled out. The permeability of the fetal membranes to a wide variety of substances, including proteins of molecular weight up to 150,000, has been reported (146,188), so transfer across the fetal membranes from maternal perfusate in the placental chamber to amniotic chamber fluid is a plausible explanation.

Krantz, et al. (123), found that when urea was added to amniotic chamber fluid at the start of an ex vivo perfusion, urea concentration in the amniotic chamber fluid declined by 85% during five hours of perfusion while measurable concentrations of urea were detected in both maternal and fetal perfusates. On the other hand, Shier, et al. (140), found no trace of bilirubin in the amniotic chamber fluid after ex vivo perfusion of the human placenta in experiments of 23 to 130 minutes duration, in which bilirubin was added to either maternal or fetal perfusate at the start of the experiment.

Partition Coefficients of Test Substances and Relationship Between Physical Properties and Placental Disposition of Test Substances

The partition coefficients of the test substances determined between octanol and the perfusate used in these experiments are listed in table XX. Also included in table XX are the molecular weights of each compound; pKa values as reported in the literature; the percent of each compound unionized at pH 7.4, which was the pH of the perfusate; and the value of the octanol/perfusate partition coefficient which assumes partitioning between the two phases occurs only for the unionized species of the compound (corrected octanol/perfusate partition coefficient).

Partitioning of ANT from the pH 7.4 perfusate into octanol was the greatest, followed in decreasing order, by ASL, SDM, and SNL. The octanol/perfusate partition coefficients determined for BSP, GLU, and DEX were not significantly different than zero. The rank order is changed if only the partitioning of the unionized species is considered, to SDM followed by ANT, ASL, and SNL.

As previously discussed, there were no significant differences in clearances of the test substances between either maternal or fetal perfusate and the placenta.

With regards to placental uptake of the test substances, there appears to be no correlation between the relative uptake of the test substances and the rank order of partition coefficients, with the possible exception that partition coefficient may explain the observation that SNL and SDM were not taken up to as great an extent as was ANT.

TABLE XX

Physical Properties of Test Substances

Compound	Molecular Weight	pKa[a]	Percent[b] Unionized at pH 7.4	Octanol/Perfusate[c] Partition Coefficient	Corrected[d] Octanol/Perfusate Partition Coefficient
				Mean ± S.D.	
ANT	188	1.4[e]	∿100	2.36 ± 0.021	2.36
ASL	214	∿9.0[f]	97.6	0.659± 0.009	0.675
SDM	310	5.9[g]	3.07	0.402± 0.039	13.3
SNL	172	10.4[h]	>99.9	0.139± 0.016	0.139
BSP	838	<1	∿0	0.045± 0.009[i]	---
GLU	180	---	100	-0.054 ± 0.024[i]	---
DEX	$4x10^5$ [j]	---	100	-0.046 ± 0.010[i]	---

[a] Negative log of acid dissociation constant.
[b] $1/[1 + 10^{(7.4-pKa)}]$ for acids, $1/[1 + 10^{(pKa-7.4)}]$ for bases, times 100.
[c] Results of these experiments, mean of three determinations.
[d] Experimentally determined partition coefficient divided by percent unionized at pH 7.4, times 100.
[e] (190)
[f] (191)
[g] (192)
[h] (193)
[i] Not significantly different than zero by t test (P>0.10).
[j] Average molecular weight.

Concentration Gradients

Steady state was not reached in any of the experiments for the drugs studied. A tendency toward the maintenance of a maternal to fetal concentration gradient was evident in all experiments for the drugs and for GLU (figures 35-40). The tendency toward a maternal to fetal gradient is especially obvious in the case of placenta 54 in which the test substances were initially added to the fetal reservoir. After three hours of perfusion, concentrations of ANT, BSP, and GLU in the maternal perfusate exceed concentrations in the fetal perfusate, and concentrations of SDM and ASL are increasing in the maternal perfusate, while decreasing in the fetal perfusate.

The maintenance of maternal to fetal gradients during ex vivo perfusion of the human placenta has been shown by other investigators for glucose (123,138), fructose (123), urea (123), creatinine (123), norethindrone (124), Medroxyprogesterone (124), and dydrogesterone (124), all of which are compounds believed to cross biological membranes by passive or facilitated diffusion. On the other hand, such compounds which are believed to be actively transported across biological membranes as l-amino acids, ascorbic acid, and free fatty acids (123,126,135,145), when studied by ex vivo perfusion of the human placenta, were shown to maintain concentration gradients in the fetal to maternal direction.

A recent review (27) lists fetal to maternal concentration ratios after equilibration, for 45 drugs, each of which was administered to the mother prior to delivery. It is of interest that only one human study reported fetal to maternal concentra-

tion ratios above 1.0, and ten others reported ratios ranging from 0.9 to 1.0. The remaining studies reported fetal to maternal concentration ratios well below 1.0.

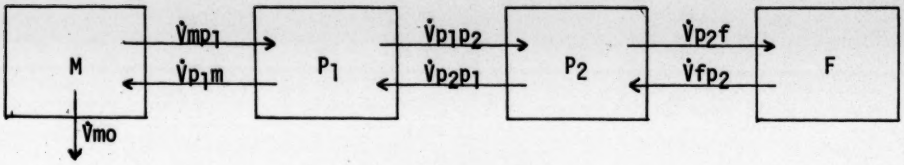
The establishment of a concentration gradient in either direction is an unexpected result of ex vivo perfusion in a closed system if the mechanism of transfer is diffusion (simple or facilitated), and if the perfusates employed in circuits on either side of the placenta were identical. At steady state in a closed system, the rate of transfer in the fetal to maternal direction is equal to the rate of transfer in the maternal to fetal direction regardless of the kinetic model employed to describe the system.

One possible model which may describe drug transfer across the placenta is illustrated in figure 42. On application of this model to ex vivo perfusion in a closed system, M would represent the maternal perfusate, F would represent the fetal perfusate, and $\dot{V}mo$ would be zero. After addition of a substance to either the maternal or fetal perfusate, the system would eventually come to equilibrium such that $dAm/dt = dAp_1/dt = dAp_2/dt = dAf/dt = 0$. Under such conditions, the equations listed in figure 42 will yield the relationship:

$$Cf/Cm = (\dot{V}mp_1\dot{V}p_1p_2\dot{V}p_2f)/(\dot{V}p_1m\dot{V}p_2p_1\dot{V}fp_2) \qquad [13]$$

If transfer between compartments takes place by diffusion alone, clearances of the substance in either direction between any two compartments will be equal; i.e., $\dot{V}mp_1 = \dot{V}p_1m$, $\dot{V}p_1p_2 = \dot{V}p_2p_1$, and $\dot{V}p_2f = \dot{V}fp_2$; and $Cf/Cm = 1$. The fact that these diffusional clearances in either direction between any two compartments will

MODEL



EQUATIONS

$$dAm/dt = -\dot{V}mp_1Cm + \dot{V}p_1mCp_1 - \dot{V}moCm$$

$$dAp_1/dt = \dot{V}mp_1Cm - (\dot{V}p_1m + \dot{V}p_1p_2)Cp_1 + \dot{V}p_2p_1Cp_2$$

$$dAp_2/dt = \dot{V}p_1p_2Cp_1 - (\dot{V}p_2p_1 + \dot{V}p_2f)Cp_2 + \dot{V}fp_2C_f$$

$$dAf/dt = \dot{V}p_2fCp_2 - \dot{V}fp_2Cf$$

SYMBOLS

M = the maternal compartment,

P_1 = the placental compartment in contact with M,

P_2 = the placental compartment in contact with F,

F = the fetal compartment,

$\dot{V}ij$ = the clearance of substance from compatment i to compartment j,

i or j = M, P_1, P_2, or F, $i \neq j$,

$\dot{V}mo$ = the elimination clearance of the substance from M,

Ai = the amount of the substance in compartment i,

dAi/dt = the rate of change of Ai with time (t),

Ci = the concentration of the substance in compartment i.

ASSUMPTIONS

1. All compartments are well mixed.
2. Transfer to amniotic fluid is negligible.

Figure 42. One possible model to describe kinetics of placental transfer.

be equal may be verified by reference to equations 4 and 5 in the Introduction. The ratio Cf/Cm will be different than 1 only if, in addition to diffusion, there is another transfer process, such as active transfer or filtration, which would cause clearances between any two compartments to be different in opposite directions.

The ex vivo perfusion experiments in these studies as well as those reported by other investigators, experienced fetal to maternal fluid flow. Those molecules small enough to pass through aqueous channels along with water will be transferred from the fetal perfusate, through the placenta, to the maternal perfusate by this process of filtration, which will be occurring simultaneously with the bidirectional transfer process of diffusion. Each of the clearances in the fetal to maternal direction will therefore be larger than the clearance in the opposite direction between the same two compartments by a factor equal to the filtrational clearance. The consequence of these unequal clearances as applied to the model presented in figure 42 will be that at steady state, the concentration of the substance in the fetal perfusate will be less than its concentration in the maternal perfusate; i.e., the ratio Cf/Cm of equation 12 will be less than unity since the denominator will be numerically greater than the numerator. Substances which are actively transported from the maternal to the fetal side of the placenta, at steady state, may exhibit fetal perfusate concentrations higher than maternal perfusate concentrations if the coefficients of the active transfer process exceed the diffusional and filtrational clearances in the fetal to maternal

direction.

The conclusions drawn from the model presented in figure 42 may also result from other models for placental transfer constructed with additional compartments, when the system is in steady state.

The existence of fetal to maternal concentration ratios less than unity in vivo may be the result of a multitude of factors, among which may be a higher affinity for binding to maternal plasma proteins than to fetal plasma proteins, biotransformation by the fetus or placenta, excretion from the fetus into amniotic fluid and subsequent transfer from the amniotic fluid directly into maternal tissues. However, transfer from the fetus through the placenta to the mother by in vivo filtration may also be a contributing factor to the frequent observations of maternal to fetal gradients for drugs.

If the model illustrated in figure 42 is employed to illustrate drug disposition in a pregnant woman and her products of conception after an intravenous dose of a substance into the mother, M would represent the distribution space in the mother, F, the distribution space in the fetus, P_1 and P_2, the distribution spaces within the placenta, and $\dot{V}mo$, the total excretory and metabolic clearance of the substance from the mother. If it is further assumed that there is no difference in protein binding affinities or red cell distribution of the substance in maternal and fetal blood, no metabolism by the placenta or fetus, and distribution in the amniotic fluid is negligible; at some time after the substance is administered equilibration (pseudo-distribution equilibrium) will occur in which $dAm/dt < 0$,

$dAp_1/dt < 0$, $dAp_2/dt < 0$, $dAf/dt < 0$, and Cf/Cm will be constant. Because the substance is being eliminated from the mother, it follows from the inequalities above that $\dot{V}fp_2Cf > \dot{V}p_2fCp_2$, $\dot{V}p_2p_1Cp_1 > \dot{V}p_1p_2Cp_1$, and $\dot{V}p_1mCp_1 > \dot{V}mp_1Cm$. If transfer between compartments takes place by diffusion alone, clearances in opposite directions between any two compartments are equal and $Cf > Cp_2 > Cp_1 > Cm$, resulting in the ratio Cf/Cm greater than unity. If the process of filtration occurs in the fetal to maternal direction simultaneously with diffusion in both directions so that clearances in the fetal to maternal direction are greater than clearances between the same compartments in the opposite direction, the concentration of the substance in fetal blood may be greater than, equal to, or less than concentration in the maternal blood during equilibration, depending on the magnitudes of the clearances.

Although others have recognized fetal or placental metabolism of drugs (194), and maternal-fetal differences in protein binding affinities (81,194) as contributing factors to Cf/Cm ratios less than unity *in vivo*, and some authors have postulated fetal to maternal active transport mechanisms (195), the role of fetal to maternal filtration as a transfer mechanism limiting the fetal concentration of drugs has not previously been discussed. The possible role of filtration in the maternal to fetal direction was considered and ruled out in a study of sodium and antipyrine transport across the human placenta by McGaughey, *et al.* (101), who did not consider the possibility of fetal to maternal filtration.

Kinetics of Placental Drug Transfer

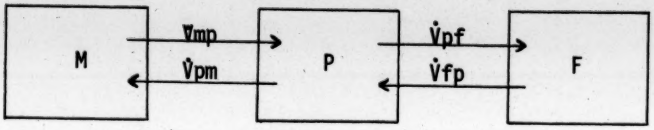
Although various authors have proposed models to describe transfer of substances across the placenta under steady state conditions (30,112,196-200), a description of placental uptake in such models is not necessary.

The kinetics of placental transfer under non-steady state conditions have been considered by several authors (28, 194,201,202). In such models, the placenta has been considered as a thin membrane in which uptake of the substance under consideration is negligible, or as a single, well-stirred compartment, in which the possibility of placental uptake is acknowledged.

In a paper dealing with factors affecting equilibration of drugs between mother and fetus, Dawes (201) discusses the effect of placental volume. The author points out the importance of taking placental volume into account when considering the kinetics of equilibration, especially when the size of the placenta is large in comparison with the size of the fetus, as occurs early in gestation.

In his consideration of the placental tissues which take up a drug, Dawes represented these tissues as a single well-stirred compartment which could either be in series, or in parallel with the placental exchange membranes. Dawes concludes that the most likely arrangement is one in which the placental exchange membranes are identical with the tissues which take up the drug, and equilibration occurs simultaneously between these tissues and the maternal and fetal blood within the placenta. This model proposed by Dawes is illustrated in figure 43 along

MODEL



EQUATIONS

$$dAm/dt = -(\dot{V}mp/Vm)Am + (\dot{V}pm/Vp)Ap$$

$$dAp/dt = (\dot{V}mp/Vm)Am - (\dot{V}pm/Vp)Ap - (\dot{V}pf/Vp)Ap + (\dot{V}fp/Vf)Af$$

$$dAf/dt = (\dot{V}pf/Vp)Ap - (\dot{V}fp/Vf)Af$$

SYMBOLS

M = the maternal compartment,

F = the fetal compartment,

P = the placental compartment,

$\dot{V}ij$ = the clearance of the substance from compartment i to compartment j,

i or j = M, P or F, $i \neq j$

Vi = the volume of distribution of the substance in compartment i,

Ai = the amount of the substance in compartment i,

dAi/dt = the rate of change of Ai with time (t).

ASSUMPTIONS

1. All compartments are well mixed.

2. Transfer to amniotic fluid is negligible.

Figure 43. Model to describe placental drug transfer kinetics proposed by Dawes.

with equations describing the model and the assumptions.

The results of the experiments presented in this study suggest that the placenta cannot be represented kinetically by the model proposed by Dawes. The equations describing Dawes' model were used to design the analog computer circuit illustrated in figure 44. The parameters were given the values listed in figure 44 to attempt the simulation of the ANT data from placentas 50 and 54. The assumptions made in choosing these parameter values are as follows:

1) All volumes and clearances are constant with time;

2) Clearances into the placenta from either circuit are equal to clearances in the opposite directions and are numerically equal to the perfusion rates for the circuit;

3) Volumes of distribution in the maternal and fetal compartments are equal to the total perfusate volumes in each reservoir and in arterial and venous circuits;

4) The placental distribution volume is equal to the volume of placental water, i.e., 0.85 l/Kg wet placental weight (153); and

5) The initial amounts are the amounts of ANT calculated from perfusate concentrations at the start of each experiment.

The computer drawn curves for concentrations in maternal and fetal perfusates, and the fraction of amount in the placenta with time are traced in figures 45 and 46 in which the data from placentas 50 and 54 are also plotted.

Comparison of the computer drawn curves, with the data, show that the model, with the parameters chosen falls far short of simulating the data. The theoretical curves show that

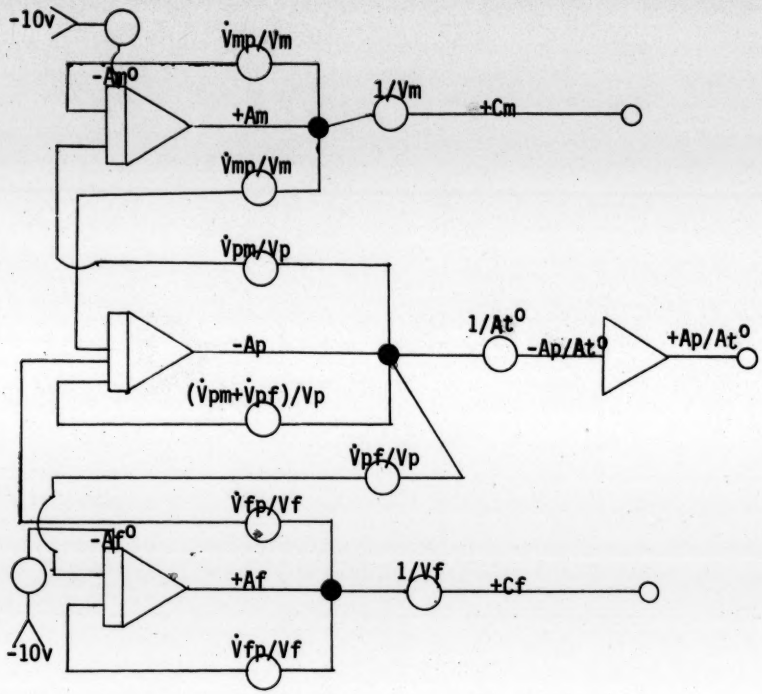


Figure 44. Analog computer circuit diagram for the solution of equations for the model illustrated in figure 43. The parameters employed to draw the curves traced in figures 45 and 46, are as listed below.

PARAMETER		VALUE	
		FIGURE 45	FIGURE 46
Initial amount in maternal compartment (Am^o),	mM	1.59	0
Initial amount in fetal compartment (Af^o),	mM	0	1.43
Total initial amount in the system (At^o),	mM	1.59	1.43
Maternal compartment volume (Vm),	l	1.75	1.25
Fetal compartment volume (Vf),	l	1.09	1.59
Placental compartment volume (Vp),	l	0.578	0.765
Clearance from maternal to placental compartment ($\dot{V}mp$),	l/min	0.600	0.600
Clearance from placental to maternal compartment ($\dot{V}pm$),	l/min	0.600	0.600
Clearance from fetal to placental compartment ($\dot{V}fp$),	l/min	0.114	0.114
Clearance from placental to fetal compartment ($\dot{V}pf$),	l/min	0.114	0.114

Magnitude Scaling: 1 mM = 6 v; 1 l = 0.6; 1 mM/l = 10 v.
Time Scaling: 1 min = 1 sec computer time
Tyrotek Aid III analog computer used in these studies (Tyrotek Corp., Moraga, Ca.).

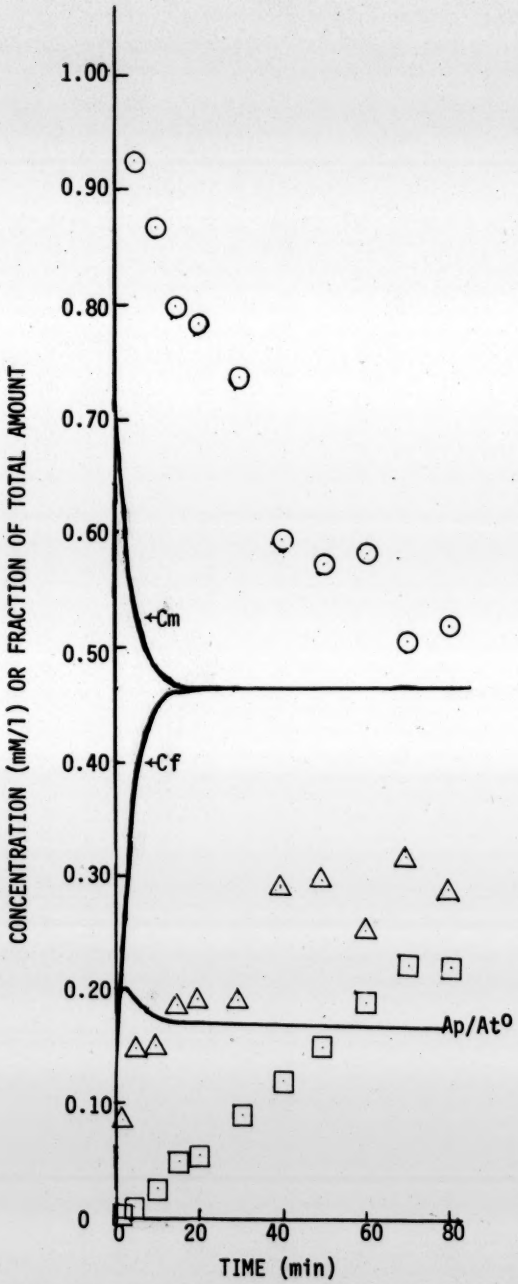


Figure 45. Comparison of computer drawn curves with ANT data of placenta 50. KEY: Maternal perfusate concentrations; Cm, computer drawn curve, (⊙), data. Fetal perfusate concentrations; Cf, computer drawn curve, (⊡), data. Fraction of total amount in placenta; Ap/At^{o}, computer drawn curve, (△), data.

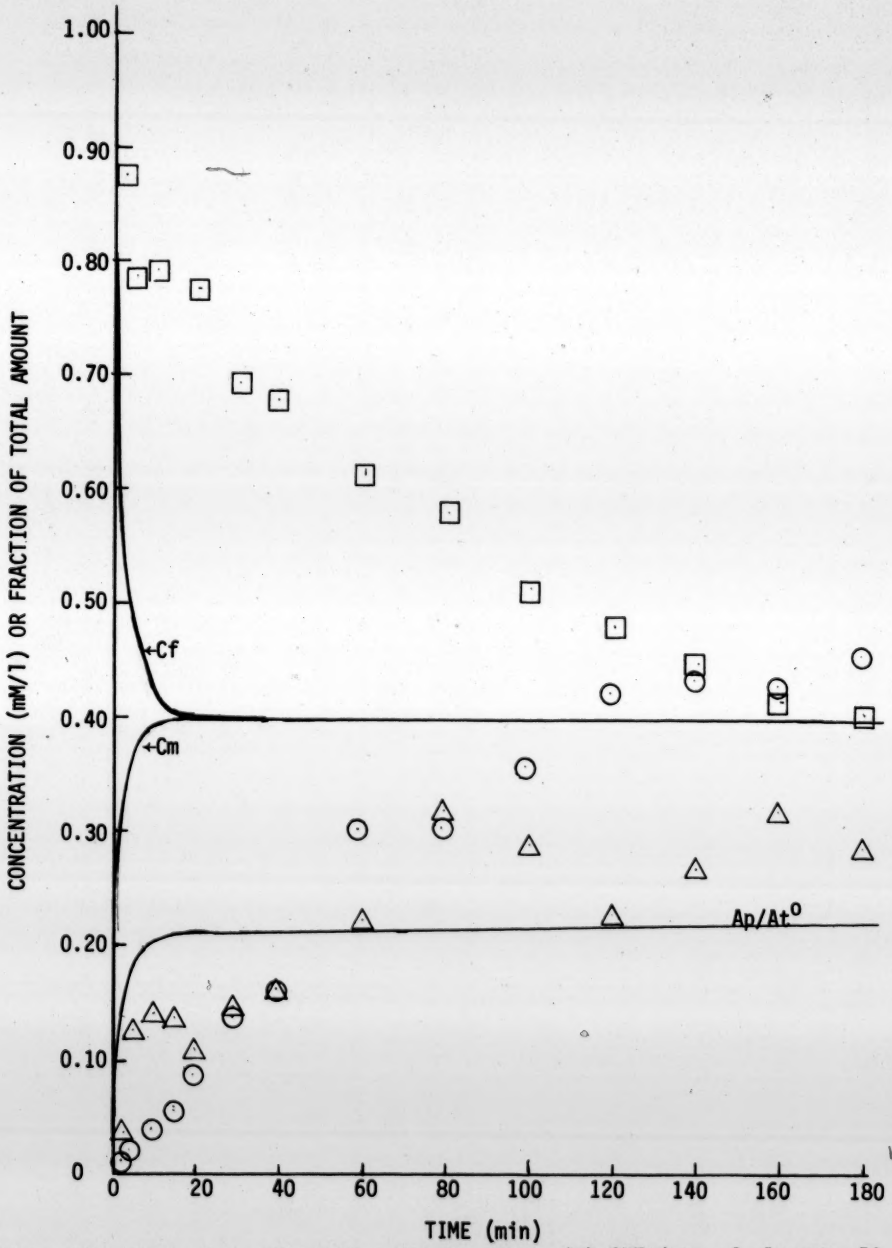


Figure 46. Comparison of computer drawn curves with ANT data of placenta 54. KEY: Maternal perfusate concentrations; Cm, computer drawn curve, (⊙), data. Fetal perfusate concentrations; Cf, computer drawn curve, (⊡), data. Fraction of total amount in the placenta; Ap/At^{o}, computer drawn curve, (△), data.

equilibrium is attained in less than 40 minutes in both cases while the data suggests that equilibrium may be approached for placenta 54 only after 160-180 minutes.

Further attempts to simulate the data with Dawes' model were attempted by each of the following changes: 1) simulation of the fetal to maternal fluid flow which occurred in these experiments, thus having variable distribution volumes and different opposing clearances; 2) increasing the volume of distribution of the placental compartment; and 3) reducing the values of the clearances. Of these changes, only when the clearances were reduced in value did the time for equilibration increase significantly. When all the clearances were reduced to less than 10% of the perfusion rates, the theoretical curves came close to simulating the experimental data. However, this result would indicate that the efficiency of antipyrine extraction from the maternal or fetal circuit into the placenta would be less than 10%. Schneider, et al., in their ex vivo studies of steady state antipyrine transfer across the perfused human placenta (204) estimate that this efficiency is in the range of 50-80%, which is a more likely figure considering the importance of this organ for essential maternal-fetal exchange.

In light of this discussion, a more realistic kinetic description of placental drug transfer is one in which additional compartments are added to account for the diffusional limitation. The least complex model which might be appropriate is that illustrated in figure 42, in which clearances between the maternal or fetal circuits and the placenta are flow limited, but clearances between the placental compartments are diffusion

limited. The data from these experiments are insufficient to confirm this model as there is no basis for assigning values to the various parameters of the model. The effect of the additional compartment, however, would be to increase the time for equilibration.

The concept of an intraplacental diffusion limitation to placental transfer is not new. Faber, et al. (203,205), from their perfusion studies of the rabbit placenta in situ, report that the greatest resistance to diffusion lies within the inner layers of the placenta between the syncytiotrophoblast and the fetal capillary endothelium.

Conclusions

1. All placentas were metabolically active but showed evidence of partial viability loss during all or part of the perfusion period.

2. There were no qualitative differences in the relative disposition rate characteristics of the substances studied in different experiments in spite of differences in viability of the placentas.

3. Clearances between the placenta and the maternal or fetal circuit, for all the substances studied, were limited by perfusate flow rate, rather than by permeabilities of the test substances. Flow rate limited clearance is evidenced by the observation that no differences existed in the initial rates of disappearances of the various substances from the reservoir to which they were initially added simultaneously, even though the test substances varied in their physicochemical properties.

4. Initial rates of appearance of the various sub-

stances, in the circuit opposite to which they were initially added varied for the test substances, apparently dependent on the degree of uptake by and/or diffusional resistance through the placental tissues.

5. Placental uptake of BSP and ASL was much higher than expected solely on the basis of lipid solubility and degree of ionization of the compounds. This greater uptake is possibly due to tissue binding.

6. Transfer of small amounts of the test substances into amniotic chamber fluid occurred, either from maternal perfusate across the fetal membranes, or from placental tissue across the chorionic plate and the overlying amnion.

7. The tendency for attainment of a maternal to fetal gradient of the test substances was observed in each experiment and may be explained as a consequence of simultaneous fetal to maternal filtration and bidirectional diffusion. This mechanism may explain in part the common in vivo findings of fetal to maternal plasma concentration ratios after equilibration of less than unity.

8. The data from these experiments show that models which represent the placenta as a thin membrane or as a single, well-stirred compartment, are not adequate to describe the kinetics of placental drug transfer in the ex vivo preparation, The kinetics of placental transfer may be better described by models which represent the placenta as two or more compartments, however, the data from these experiments were inadequate for determination of a more complex model.

SUGGESTIONS FOR FURTHER RESEARCH

The use of the ex vivo perfused human placenta preparation holds much promise in the study of placental functions under conditions similar to those which exist in vivo. Until the problems encountered by the development of increased capillary permeability with time are solved, the use of this preparation for quantitative studies of placental transfer and metabolism will be difficult.

To this end, the most immediate problem to be solved is prevention of the increasing fetal capillary permeability encountered in the experiments reported in this work. It should be possible to solve this problem in one or both of the following ways:

First, by improving procedures for preservation of the organ during the period between delivery and perfusion of the placenta. Recent studies in the preservation of human organs for subsequent transplantation may aid in the solution of this problem (167).

Secondly, the composition of the perfusion medium may be a factor in determining the duration of placental viability. In these experiments, the use of blood, or plasma, was avoided in order that the effect of plasma protein binding of the test substances would not influence the rate of placental transfer. It has been suggested, however, that plasma globulins are an essential ingredient of the fetal perfusate to prevent increases in capillary permeability during placental perfusion (121).

With improvement in the viability of the organ and integrity of the membranes with time, a precise study of drug

transfer across the placenta and into the amniotic fluid, and of placental drug uptake, may be undertaken in order to shed more light on placental transfer mechanisms and to better define a kinetic model for placental transfer.

Also, placental drug metabolic reactions, which have been shown to occur in placental homogenates, may be studied in the ex vivo perfused human placenta, the results of such experiments may, to a greater degree, reflect the importance of these reactions in vivo.

These studies would better define the effect of the placenta on the rate and extent of drug transfer to the fetus.

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